Abstract

MULTIPLE FACTORS INFLUENCE THE STRENGTH OF INTRAGUILD INTERACTIONS

by Jon M. Davenport

April, 2011

Department Chair: Jeff McKinnon, Ph.D.

DEPARTMENT OF BIOLOGY

Species engaged in intraguild predation (IGP) not only compete for the same food resources but can also eat each other. In some cases, a predator species in a higher trophic position (i.e., a top predator) can eat a predator species with an intermediate trophic position (i.e., an intermediate predator) but the intermediate predator cannot consume the top predator. An important question in ecology is how can intermediate predators persist with top predators that both eat and compete with them? I examined how environmental complexity (food web complexity, variability in the kinds of environmental cues that elicit behavioral/morphological responses of predators, and habitat complexity) affects the strength of IGP within temporary ponds. To do this, I conducted experiments in artificial ponds and focused on interactions between larval dragonflies (*Anax* spp., a top predator) and larval salamanders (*Ambystoma opacum*, an intermediate predator). Salamander survival was consistently reduced by dragonflies but the effect of dragonflies on salamander survival was enhanced in structurally complex environments that facilitated the ambush hunting style of dragonflies. The provision of either more prey individuals or alternative prey species did not diminish the effect of dragonflies on salamander survival. Salamanders did alter their behavior and morphology in response to dragonflies in ways that reduced their mortality risk to dragonflies. A high abundance of

conspecifics, however, reduced the extent to which salamanders alter their morphology. Larval salamander growth, a trait that affects fitness of adults, was also affected by environmental complexity. Although the abundance and kinds of prey available had no effect on salamander survival, they enhanced the positive effect dragonflies had on salamander growth by ensuring survivors had more high quality food to eat. Morphological responses of salamanders to dragonflies did not cause a reduction in the foraging ability of salamanders. These results support the hypothesis that environmental complexity can alter the strength of IGP interactions. Although I have not found support for theory which attempts to explain how intermediate predators can persist in food webs with top predators, the results from my dissertation highlight the environmental conditions that promote the likelihood of coexistence between predators involved in IGP.

MULTIPLE FACTORS INFLUENCE THE STRENGTH OF INTRAGUILD INTERACTIONS

A Dissertation Presented To

The Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by

Jon M. Davenport

April, 2011

MULTIPLE FACTORS INFLUENCE THE STRENGTH OF INTRAGUILD INTERACTIONS

by

Jon M. Davenport

APPROVED BY:

DIRECTOR OF DISSERTATION:_____
David Chalcraft, Ph.D.

COMMITTEE MEMBER:_____
Nick Haddad, Ph.D.

COMMITTEE MEMBER:_____
Claudia Jolls, Ph.D.

COMMITTEE MEMBER:_____
Trip Lamb, Ph.D.

COMMITTEE MEMBER:_____
Heather Vance-Chalcraft, Ph.D.

CHAIR OF THE DEPARTMENT OF BIOLOGY:

_____
Jeff McKinnon, Ph.D.

DEAN OF THE GRADUATE SCHOOL:

_____
Paul J. Gemperline, Ph.D.

ACKNOWLEDGMENTS

Many people have helped in some capacity or another in the making of this dissertation. First, I would like to thank my advisor, David Chalcraft, for providing support and pushing me to think broader and more passionately about community ecology. I am also indebted to him for fostering my appreciation and now obsession for North Carolina barbeque. I would like to thank my committee: Nick Haddad, Claudia Jolls, Trip Lamb, and Heather Vance-Chalcraft for guidance and feedback over the last six years.

I am especially indebted to my lab mates, both past and present, for their help setting up experiments, good conversation and general tolerance of me over the years. They are as follows: Natalie Amoroso, Leah Connell, Robbie Deans, Jason Hernandez, Freddy Herrera, Scott Jones, Lauren McCarthy, Alyssa Miller, Matt Prentice, Tracy Rogers, Cliff Ruehl and Chuck Williams. Thanks also goes out to the awesome group of graduate students in the Biology department that have helped indulged in libations and provided me with a strong sense of family: Dave Beamer, Adam Davis, Jesse Delia, Chris Hamilton, Richard Hanna, Brent Hendrixson, Jennifer Ness, Krissy Rehm, Matt Walker, and Jennifer Weber.

I also would like to give thanks to Gene Oakley of the ECU Physics department for his help building my aquatic photo chamber. I am grateful to the staff at the Queen Anne's Revenge for providing me with space at the West Research Campus during my experiments. I also thank Ken Alexander, Dave Beamer, Jesse Delia, Julie Marek, Jennifer Moore, Michele Pierotti, and Justin Yeager for help with experiments over the last six years. I would also like to thank faculty and staff in the ECU Biology Department who have offered advice, assisted with administrative paperwork and acted as a sounding board for my ideas: Barbara Beltran, Jason Bond, Grant Gardner, Anthony Overton, Enrique Reyes, Jean-Luc Scemama, Kyle Summers, and Terry West.

Financial support was provided for me and my dissertation research by the ECU Department of Biology, a National Science Foundation grant (DEB -0716558) awarded to David Chalcraft, and a grant from the North Carolina Herpetological Society awarded to me.

Finally, I would especially like to thank family for their support of my continuing education. Specifically, I thank my parents for always encouraging me to investigate the natural world around me, to always play outside, and for enduring my fascination with all things creepy and crawly. I also would not have been able to weather the storm that is graduate school without the love and companionship of my wife, Jenny. She has and continues to help me to become a better scientist and a better person and for that I will forever be in her debt. I also thank my baby girl, Leoria, for rekindling my childhood excitement and wonder about the big world around us. Leoria has also provided me with the extra incentive to finally finish up this leg of the journey and get a job.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: Background

Introduction

One type of interaction within a food web is intraguild predation (IGP) involving at least two predator species that potentially compete for the same food resource (shared prey) and where at least one of the predator species (top predator) can consume the other predator species (intermediate predator) (Holt and Polis 1997). IGP differs from conventional predation since top predators reduce the abundance of competitors; or also differs from conventional competition as at least one of the predator species can directly consume the other predator species (Polis et al. 1989). Although top predators should prevent intermediate predators from persisting with them by reducing the abundance of intermediate predators via competition and predation (Polis et al. 1989), IGP appears to be quite common in nature (Arim and Marquet 2004). This begs the question, what allows intermediate predators to persist with top predators?

Seminal work by Holt and Polis (1997) provided the first theoretical explanations to understand the stability of IGP dynamics. Several predictions emerged from initial theory for the necessary conditions of IGP coexistence: 1) intermediate predators must be superior competitors for a shared prey resource, 2) top predators should gain substantial benefits from consumption of intermediate predators, 3) the top predator will positively influence the abundance of shared prey resources indirectly via consumption of intermediate predators, and 4) as productivity increases, dominance will shift from intermediate predators to top predators with coexistence at intermediate levels (Holt and Polis 1997). Theory explicitly predicts that intermediate predators should dominate in low productivity environments due to their superior competitive ability while top predators should dominate in high productivity environments since top predators will become numerically dominant with more resources (and be able to exert stronger predation pressure on

intermediate predators). Thereby, coexistence is predicted to occur during intermediate levels of productivity when neither top nor intermediate predators can exclude the other (Holt and Polis 1997).

Ecologists studying IGP, however, have found that observations from natural settings do not match theoretical predictions (Cortwright 1988, Wissinger 1989, Olsen et al. 1995, Morin 1999, Diehl and Fessel 2000, 2001, Rosenheim 2007). Some studies have found that top predators are superior competitors (Wissinger and McGrady 1993, Diehl 1995), with top predators being larger and often more aggressive than intermediate predators (Johansson 1993, Fedriani et al. 1999, Wissinger et al. 1999). Top predators often interfere with intermediate predators indirectly by causing intermediate predators to decrease foraging activity (Huang and Sih 1991, Gustafson 1993, Walls and Williams 2001). Therefore, ecologists have now begun to expand the simple theoretical IGP models to reconcile this discrepancy between IGP theoretical predictions and IGP empirical work (Amaresekare 2007a,b, Borer et al. 2007). The expanded IGP models have led to a whole new round of theoretical predictions and provide ecologists with a fruitful avenue to investigate persistence and stability of IGP food webs (Abrams and Matsuda 1997, Vos et al. 2004, Daugherty et al. 2007, Holt and Huxel 2007, Kratina et al. 2010).

The persistence of top and intermediate predators may be due to multiple mechanisms. Three possible mechanisms that could allow intermediate and top predators to co-occur include; 1) the complexity of the food web in which an intermediate predator is embedded (e.g., presence of alternative prey for top predators may reduce predation risk for intermediate predators), 2) the induction of intermediate predator phenotypic traits (behavior, morphology, and life history) in response to top predators as mediated by conspecific densities in the environment, and 3) the amount and type of habitat complexity in the environment which likely influences the overall

encounter rates between top and intermediate predators or foraging efficiency of top predators on intermediate predators. Many studies have documented intraguild predation and although mechanisms have been proposed to explain the coexistence of intermediate and top predators, many have yet to be tested experimentally (Holt and Polis 1997).

Study System

Temporary ponds present a model system for population and community ecologists. Temporary ponds can vary in size (from small phytotelmata to large playas in the American southwest) and can support a diverse ensemble of invertebrates and vertebrates (amphibians) (Wilbur 1997). Ponds form discrete boundaries (e.g., pond-terrestrial interface) where communities of organisms can interact directly and indirectly in a myriad of ways (Wilbur 1997). Competition among species begins to strengthen as ponds fill, since nutrients are released into the pond and species begin to accumulate. Predation also plays an important role in structuring pond communities as more predators arrive to exploit herbivores at lower trophic levels (Wilbur 1972, Morin 1983, Chalcraft and Resetarits 2003). Ponds in nature exist along a hydroperiod gradient, ranging from permanent ponds that dry irregularly and can be a relatively stable environment, to temporary ponds that dry up regularly and add a level of environmental uncertainty for colonizing species (Wilbur 1987, Wellborn et al. 1996). All of these attributes of the temporary pond study system provide ecologists with an excellent opportunity to develop hypotheses regarding the regulation of natural communities.

Within temporary pond systems, I chose to examine factors influencing the strength of interactions between darner dragonfly larvae of the genus *Anax*, a top predator, and larvae of the marbled salamander (*Ambystoma opacum*), an intermediate predator. Both species can be found

separately and together in temporary pond communities in the eastern half of the US and both consume small invertebrates and larval anurans. Some aquatic top predators, such as fish, are absent from the temporary pond communities due to the ephemeral nature of temporary ponds. Fish are voracious predators of both larval amphibians and dragonflies thus restricting the distribution of many amphibian and dragonfly species to temporary pond communities (Crowder and Cooper 1982, Petranka 1983, Morin 1984, Chalcraft and Resetarits 2003). The exclusion of fish from temporary pond communities provides the opportunity for invertebrates (e.g., larval dragonflies such as *Anax*) and certain amphibian species (e.g., predatory salamanders) to become the important predators in these communities (Wellborn et al. 1996). The marbled salamander, *Ambystoma opacum*, and the dragonfly larvae, *Ana*x, found locally in North Carolina present an opportunity to explore the dynamics of IGP in aquatic food webs. *A. opacum* arrives earlier (fall and early winter) than any other pond breeding amphibians and typically achieves a large size by the time other amphibians arrive. This size advantage has led to the larvae of *A. opacum* being regarded as important predators of larval frogs during the spring (Morin 1995, Petranka 1998, Chalcraft and Resetarits 2003: Figure 1). Large, overwintered larval *Anax* can also be found in the same ponds where *A. opacum* lay their eggs in the fall if the pond had not completely dried. This size advantage allows *Anax* to be an effective predator on larval amphibians and other prey (Crumrine 2005). Both *Anax* and *A. opacum* consume small invertebrates and small larval anurans (e.g., *Pseudacris*, *Rana* and *Bufo*) during the spring, but *A. opacum* are unable to eat some of the larger prey that *Anax* can eat (e.g., overwintered *Rana* spp.). It is unclear how much dietary overlap for zooplankton occurs between *Anax* and *A. opacum*.

Description of Research

Food Web Complexity

Since food web topology can overwhelm strong effects directly and indirectly on the strength of species interaction (O'Gorman et al. 2010), my first objective was to quantify the performance of an intermediate predator in food webs that differed in complexity (Chapter 2). Most studies of intraguild interactions focus on a simple three species module that is less reflective of natural food webs (Yurewicz 2004, Daugherty et al. 2007, Holt and Huxel 2007). Chapter 2 investigates the effects of food web complexity (multiple predators, shared and unshared prey species at various abundances) on the strength of intraguild interactions in speciose food webs containing intraguild interactions (Polis 1991).

Persistence between one predator and a prey species has been repeatedly shown to be promoted by the availability of alternative species of prey (Paine 1966, Morin 1981). Therefore, it seems reasonable to expect that the presence of alternative prey for top predators could promote the persistence of top and intermediate predators (Finke and Denno 2005). Top predators in complex food webs likely have multiple food items to consume in comparison to the intermediate predator to choose from in the same background community (Walls and Williams 2001). This increase in food items for the top predator is due to feeding at multiple trophic levels. Limited studies have simultaneously manipulated shared prey relative abundance when food webs contained both top and intermediate predators. Previous work (Polis and McCormick 1987, Wissinger and McGrady 1993, Wissinger et al. 1999) has established the varying impacts that top and intermediate predators have on a shared prey, but most have only manipulated the presence or absence of shared prey. Generally, the shared prey experiences increased survival in the presence of both predators in comparison to one predator alone.

To this end, I conducted an experiment in mesocosms where I disassembled a complex food web (with top predators, intermediate predators, shared prey and two types of unshared prey) to understand the effects of food web complexity on intermediate predator performance. I hypothesized that the more complex food webs (presence of shared or unshared prey at increased abundances) will reduce the strength of intraguild interactions (competitive and consumptive pressure on intermediate predators by top predators) and support the persistence of top and intermediate predators.

Variation in chemical cues emitted by predators and competitors

Studies have documented that prey change their phenotype (morphological and behavioral) in response to the presence of predators (Tollrian and Harvell 1999). Furthermore, changes in prey traits may also occur as a result of a change in the competitive environment that the prey is exposed to. For example, herbivorous tadpoles can change their phenotype in response to the presence of competitors and the degree of phenotypic change observed is a function of competitor density; competitor-induced phenotypic changes in herbivores were strikingly different than predator-induced changes (Relyea 2002, 2004). A trade-off between expressing a competitor- and predator-induced phenotype may exist due to phenotypes differing in how energy should be allocated for the production of different structures or behaviors (Peacor 2003, McCoy 2007, Van Buskirk et al. in press). One phenotype may require allocation of energy to defenses from a predator while the other may simply allocate more energy to increased foraging and efficiency in garnering resources. Thus, previous research indicates that it is possible for organisms to express unique phenotypes dependent on cues in the environment (Tollrian and Harvell 1999, McCoy 2007).

In ecological communities, organisms are exposed to predators and competitors concurrently and must assess their surroundings in order for particular phenotypes to be expressed. As conspecific density increases, the perceived predation risk due to a predator is lowered because there are lots of other prey that the predator could eat. Therefore, the magnitude of phenotypic responses to predators may be mediated by conspecific density (Peacor 2003). Intermediate predators may alter their phenotype in accordance to the level of competition (abundance of conspecifics) experienced in the environment but also must account for the change in abundance of conspecifics due to predation. For example, at higher abundances of competitors, an intermediate predator will express a phenotype that is efficient at exploiting resources while also assessing the risk of predation. No study has quantified how the exposure to multiple levels of conspecific density and predation cause changes in the traits of intermediate predators.

One final issue that remains unknown from prior studies examining morphological changes in prey to predators or competitors is the degree to which these changes are observed at different stages of larval development (Relyea 2003, 2007). In one of the only studies to address predator-induced traits throughout development, Relyea (2003) found that larval anurans changed their phenotype over ontogeny. No studies have documented how predators and competitors influence development of morphology throughout ontogeny. Such observations are important for multiple reasons. First, the inducement of a particular morphology early in development may preclude other types of changes later in development (Hoverman and Relyea 2007). Second, identifying the timing in which predators and competitors induce changes could indicate which developmental stages of an animal are at a greater risk of reduced competitive ability or risk to predation.

Most phenotypic plasticity studies have focused on predator-herbivore interactions; very few studies have quantified how traits of intermediate predators are altered in response to top predators (e.g., Van Buskirk and Schmidt 2000, Yurewicz 2004) or competitors. As with a prey species, modification of intermediate predator traits should occur to increase an individual's fitness in the presence of a predator (Van Buskirk 2000, Benard 2006). My first objective was to investigate if intermediate predators can respond phenotypically to cues in their environment from predators and competitors and if so how those phenotypic traits were altered through time (Chapter 3). I conducted a plasticity experiment in mesocosms to answer this objective. For my second objective, I was also interested in understanding how phenotypic changes in an intermediate predator affected its foraging efficiency and vulnerability to top predators. To this end, I conducted two experiments in mesocosms evaluating the performance of intermediate predator phenotypes from different larval environments.

First, I hypothesized that intermediate predators exposed to gradients of predation and competition will modify phenotypic traits in accordance to the risk of mortality that they experience and the intensity of competitive interactions imposed on them. Second, I hypothesized that intermediate predators phenotypes will reflect the demands imposed by both competitors and predators (i.e., changes in body and tail size reflect environmental conditions) since it is likely these interactions induce traits in opposite directions. Third, based on prior work with herbivores, I proposed that predator- and competitor-induced morphological traits will not be observed until later in ontogeny, while behavioral traits will be modified early in ontogeny. Fourth, I expected that intermediate predators with predator-induced phenotypes will experience reduced vulnerability to lethal top predators and reduced foraging efficiency relative to individuals without predator-induced phenotypes. Finally, I hypothesized that performance of

intermediate predators with competitor-induced phenotypes will be more proficient in obtaining prey items but more vulnerable to predation by top predators.

Habitat Complexity

My final factor that I evaluated was the effects of two different types of habitat complexity on the strength of interactions between top and intermediate predators (Chapter 4). Habitat complexity has been considered to be an important factor in stabilizing predator-prey interactions in natural settings (Connell 1970) because it can reduce predator foraging efficiency (Stein and Magnuson 1976) and/or it provides prey with at least some predator free space (Huffaker 1958, Babbitt and Tanner 1998). Most work examining the effects of habitat complexity on predator-prey or predator-predator interactions; however, have focused on only one aspect of habitat complexity: the amount of a one particular kind of structure present in the environment.

Studies on the effects of habitat complexity on species interactions (e.g., competition and predation) are relatively common (McCoy and Bell 1991), however, studies that incorporate the different measures of habitat complexity on species interactions are not. Habitat complexity usually varies in more than one way and therefore can be measured by more than one metric. One such way to further our understanding of the effects of habitat complexity is to investigate how multiple metrics (such as the amount of one kind of complexity and the amount of another kind of complexity) interact to affect species interactions. Different measures of habitat complexity may lead to a matrix of benefits and costs for predators and prey. Few studies have quantified how different measures of habitat complexity alter the strength of species interactions especially in complex food webs with IGP.

Finke and Denno (2002) studied the effects of habitat complexity on intraguild interactions and found that intermediate predators find spatial refuge from top predators in complex habitats. This led to increased exploitation of the shared prey resource by both predators. Shared prey may increase refuge use to escape top predators (who are assessed as being a bigger threat), but in doing so may enhance their predation risk by exposure to intermediate predators in the same refuge. Finke and Denno (2002) found that habitat complexity improved the foraging and capturing efficiency of intermediate predators (wolf spiders) on herbivore shared prey (grasshoppers) (Finke and Denno 2002). The improved foraging success of intermediate predators observed in complex habitats by Finke and Denno (2002) may explain the idea proposed by Polis and Holt (1997) that intermediate predators must have an increased competitive ability to persist with top predators. The increased efficiency, however, contradicts other studies where predators in general exhibit reduced searching and capture efficiency in complex habitats (Denno et al. 2005). This work highlights the potentially intricate way in which the effects of habitat complexity can manifest in IGP food webs.

To address how habitat complexity affects intraguild interactions, I conducted an experiment in mesocosms to examine how the effects of two different measures of habitat complexity (amount of benthic leaf litter versus amount of emergent aquatic vegetation) influence the effect of a sit-and-wait top predator (*Anax*) on fitness components of an active-foraging intermediate predator (*A. opacum*). I hypothesized that different types of habitat complexity (leaf litter vs. emergent vegetation) are not equally advantageous to intermediate predators. One kind of habitat complexity (leaf litter) will be advantageous to intermediate predators by providing intermediate predators with benthic refuges to hide from top predators. Another kind of habitat complexity (emergent vegetation) may be disadvantageous to

intermediate predators by providing top predators with perch sites therefore increasing encounter rates.

References

Abrams, P.A. and H. Matsuda. 1997. Prey adaptation as a cause of predator-prey cycles. Evolution 51:1742-1750.

Amarasekare, P. 2007a. Trade-offs, temporal variation, and species coexistence in communities with intraguild predation. Ecology 88:2720-2728.

Amarasekare, P. 2007b. Spatial dynamics of communities with intraguild predation: the role of dispersal strategies. American Naturalist 170:819-831.

Arim, M. and P. Marquet. 2004. Intraguild predation: a widespread interaction related to species biology. Ecology Letters 7:557-564.

Babbitt, K. J. and G.W. Tanner. 1998. Effects of cover and predator size on survival and development in *Rana utricularia* tadpoles. Oecologia 114:258-262.

Benard, M. F. 2006. Survival trade-offs between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). Ecology 87:340-346.

Borer, E.T., C.J. Briggs, and R.D. Holt. 2007. Predators, parasitoids, and pathogens: a cross-cutting examination of intraguild predation theory. Ecology 88:2681-2688.

Chalcraft, D.R. and W.J. Resetarits, Jr. 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? Ecology 84:2407-2418.

Connell, J.H. 1970. A predator-prey system in the marine intertidal region. I. *Balanus glandula* and several predatory species of *Thais*. Ecological Monographs 40:49-78.

Cortwright, S.A. 1988. Intraguild predation and competition: an analysis of net growth shifts in larval amphibian prey. Canadian Journal of Zoology 66:1813-1821.

Crowder, L.B. and W.E. Cooper. 1982. Habitat structural complexity and the interaction between bluegills and their prey. Ecology 63:1802-1813.

Crumrine, P.W. 2005. Size structure and substitutability in an odonate intraguild predation system. Oecologia 145:132-139.

Daugherty, M.P., J.P. Harmon, and C.J. Briggs. 2007. Trophic supplements to intraguild predation. Oikos 116:662-677.

Denno, R.F., D.L. Finke, and G.A. Langellotto. 2005. Direct and indirect effects of vegetation structure and habitat complexity on predator-prey and predator-predator interactions. Pages 211-239 *in* P. Barbosa and I. Castellanos, editors. Ecology of Predator-Prey Interactions. Oxford University Press, London, UK.

Diehl, S. 1993. Relative consumer sizes and the strengths of direct and indirect feeding interactions in omnivorous feeding relationships. Oikos 68:151-157.

Diehl, S. 1995. Direct and indirect effects of omnivory in a littoral lake community. Ecology 76:1727-1740.

Diehl, S. and M. Feissel. 2000. Effects of enrichment on three-level food chains with omnivory. American Naturalist 155:200-218.

Diehl, S. and M. Feissel. 2001. Intraguild prey suffer from enrichment of their resources: a microcosm experiment with ciliates. Ecology 82:2977-2983.

Fedriani, J.M., F. Palomares, and M. Delibes. 1999. Niche relations among three sympatric Mediterranean carnivores. Oecologia 121:138-148.

Finke, D.L. and R.F. Denno. 2002. Intraguild predation diminished in complex habitats: implications for top-down suppression of prey populations. Ecology 83:643-652.

Finke, D.L. and R.F. Denno. 2005. Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. Ecology Letters 8:1299-1306.

Holt, R.D. and G.A. Polis. 1997. A theoretical framework for intraguild predation. American Naturalist 149:745-764.

Holt, R.D. and G.R. Huxel. 2007. Alternative prey and the dynamics of intraguild predation: theoretical perspectives. Ecology 88:2706-2712.

Hoverman, J.T. and R.A. Relyea. 2007. How flexible is phenotypic plasticity? Developmental windows for the induction and reversal of inducible defenses. Ecology 88:693-705.

Huang, C. and A. Sih. 1991. Experimental studies on direct and indirect interactions in a three trophic-level stream system. Oecologia 85:530-536.

Huffaker, C. B. 1958. Experimental studies on predation: dispersion factors and predator-prey oscillations. Hilgardia 27:343-383.

Gustafson, M.P. 1993. Intraguild predation among larval plethodontid salamanders: a field experiment in artificial stream pools. Oecologia 96:271-275.

Johansson, F. 1993. Intraguild predation and cannibalism in odonate larvae: effects of foraging behaviour and zooplankton availability. Oikos 66:80-87.

Kratina, P. E. Hammill, and B.R. Anholt. 2010. Stronger inducible defences enhance persistence of intraguild prey. Journal of Animal Ecology 79:993-999.

McCoy, E.D. and S.S. Bell. 1991. Habitat structure: the evolution and diversification of a complex topic. Pages 3-27 *in* S.S. Bell, E.D. McCoy and H.R. Mushinsky, editors. Habitat Structure: the Physical Arrangement of Objects in Space. Chapman and Hall, New York, New York, USA.

McCoy, M.W. 2007. Conspecific density determines the magnitude and character of predator-induced phenotype. Oecologia 153:871-878.

Morin, P.J. 1981. Predatory salamanders reverse the outcome of competition among three species of anuran tadpoles. Science 212:1691.

Morin, P.J. 1983. Predation, competition, and the composition of larval anuran guilds. Ecological Monographs 53:119-138.

Morin, P.J. 1984. The impacts of fish exclusion on the abundance and species composition of larval odonates; results of short-term experiments in a North Carolina farm pond. Ecology 65:53-60.

Morin, P.J. 1995. Functional redundancy, non-additive interactions, and supply-side dynamics in experimental pond communities. Ecology 76:133-149.

Morin, P.J. 1999. Productivity, intraguild predation, and population dynamics in experimental food webs. Ecology 80:752-760.

O' Gorman, E.J., U. Jacob, T. Jonsson, and M.C. Emmerson. 2010. Interaction strength, food web topology and the relative importance of species in food webs. Journal of Animal Ecology 79:682-692.

Olson, M.H., G.G. Mittelbach, and C.W. Osenberg. 1995. Competition between predator and prey: resource-based mechanisms and implications for stage-structured dynamics. Ecology 76:1758-1771.

Paine, R.T. 1966. Food web complexity and species diversity. American Naturalist 100:65-75.

Peacor, S.D. 2003. Phenotypic modifications to conspecific density arising from predation risk assessment. Oikos 100:409-415.

Petranka, J.W. 1983. Fish predation: A factor affecting the spatial distribution of a stream-dwelling salamander. Copeia 1983:624-628.

Petranka, J.W. 1989. Density-dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. Ecology 70:1752-1767.

Petranka, J. 1998. Salamanders of the United States and Canada. Smithsonian Press, Washington, D.C., USA.

Polis, G.A. 1991. Complex interactions in deserts – an empirical critique of food-web theory. American Naturalist 138:123-155.

Polis, G.A. and S.J. McCormick. 1987. Intraguild predation and competition among desert scorpions. Ecology 68:332-343.

Polis, G.A. and R.D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends in Ecology and Evolution 7:151-154.

Polis, G.A., C.A. Myers, and R.D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297-330.

Relyea, R.A. 2002. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. Ecological Monographs 72:523-540.

Relyea, R.A. 2003. Predators come and go: the reversibility of predator-induced traits. Ecology 84:1840-1848.

Relyea, R.A. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. Ecology 85:172-179.

Relyea, R.A. 2007. Getting out alive: how predators affect the decision to metamorphose. Oecologia 152:389-400.

Resetarits, W.J., Jr. and J.E. Fauth. 1998. From cattle tanks to Carolina bays: the utility of model systems for understanding natural communities. Pages 133-151 *in* W.J.

Resetarists, Jr. and J. Bernardo, editors. Experimental Ecology: Issues and Perspectives. Oxford University Press, New York, New York, USA.

Rubbo, M. J., K. Shea, and J.M. Kiesecker. 2006. The influence of multi-stage predation on population growth and the distribution of the pond-breeding salamander, *Ambystoma jeffersonianum*. Canadian Journal of Zoology 84:449-458.

Scott, D.E. 1990. Effects of larval density in *Ambystoma opacum*: an experiment in large scale field enclosures. Ecology 71:296-306.

Stein, R.A. and J. J. Magnuson. 1976. Behavioral response of crayfish to a fish predator. Ecology 57:751-761.

Storfer, A. and C. White. 2004. Phenotypically plastic responses of larval tiger salamanders, *Ambystoma tigrinum*, to different predators. Journal of Herpetology 38:612-615.

Tollrian, R. and D. Harvell. 1999. The ecology and evolution of inducible defenses. Princeton University, Princeton, New Jersey, USA.

Van Buskirk, J. 2000. The costs of an inducible defense in anuran larvae. Ecology 81:2813-2821.

Van Buskirk, J. and B.R. Schmidt. 2000. Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. Ecology 81:3009-3028.

Van Buskirk, J., M. Ferrari, D. Kueng, K. Näpflin, and N. Ritter. *In press*. Prey risk assessment depends on conspecific density. Oikos.

Vos, M., A.M. Verschoor, B.W. Kooi, F.L. Wackers, D.L. DeAngelis, and W.M. Mooji. 2004. Inducible defenses and trophic structure. Ecology 85:2783-2794.

Walls, S.C. and M.G. Williams. 2001. The effect of community composition on persistence of prey with their predators in an assemblage of pond-breeding amphibians. Oecologia 128:134-141.

Wilbur, H.M. 1972. Competition, predation, and the structure of the *Ambystoma-Rana sylvatica* community. Ecology 53:3-21.

Wilbur, H.M. 1987. Regulation of structure in complex systems: Experimental temporary pond communities. Ecology 68:1437-1452.

Wilbur, H.M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology 78:2279-2302.

Wissinger, S.A. 1989. Seasonal variation in the intensity of competition and predation among dragonfly larvae. Ecology 70:1017-1027.

Wissinger, S.A. and J. McGrady. 1993. Intraguild predation and competition between larval dragonflies: direct and indirect effects on shared prey. Ecology 74:207-218.

Wissinger, S.A., H.H. Whiteman, G.B. Sparks, G.L. Rouse, and W.S. Brown. 1999. Foraging trade-offs along a predator-permanence gradient in subalpine wetlands. Ecology 80:2102-2116.

Yurewicz, K. L. 2004. A growth/mortality trade-off in larval salamanders and the coexistence of intraguild predators and prey. Oecologia 138:102-111.

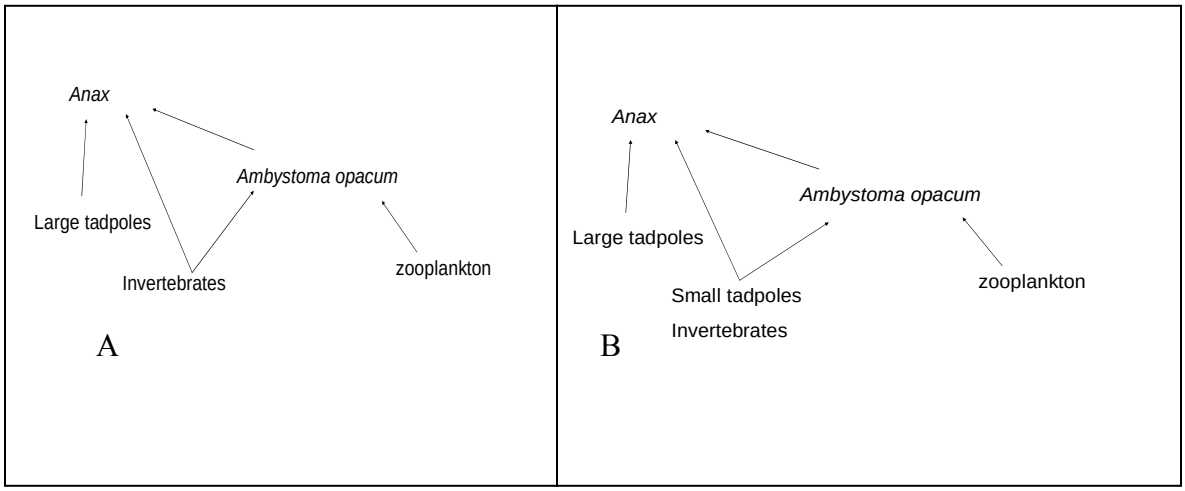


Figure 1. Temporary pond food web during the fall (A) and spring (B).

CHAPTER 2: Evaluating the Effects of Trophic Complexity on a Keystone Intermediate Predator by Disassembling a Partial Intraguild Predation Food Web

Introduction

One fascinating aspect of ecological communities is the range of complexity in the web of interactions that individual species can be found. This can be particularly true for species occupying the intermediate predator trophic position within food webs involving intraguild predation (IGP) (Polis et al.1989, Polis and Holt 1992, Holt and Polis 1997). Specifically, intermediate predators can occur in simple food webs consisting of only the intermediate predator and its prey to more complex partial IGP food webs consisting of the intermediate predator, a top predator, shared prey and unshared prey species (Daugherty et al. 2007, Holt and Huxel 2007). More importantly, intermediate predators are known to occur across many different food webs in nature (Borer et al. 2003, Arim and Marquet 2004, Thompson et al.2007, Hunter 2009). For example, I have observed larvae of marbled salamanders (*Ambystoma opacum*), an intermediate predator, in natural pond food webs that differ in complexity due to the occurrence of top predators and prey shared by top and intermediate predators (Figure 1). Although a limited amount of work has examined how prey species perform in food webs that vary in trophic complexity (e.g., presence/absence of intraguild interactions, number of predator species present;Borer 2002, Finke and Denno 2004, Carey and Wahl 2010), there is even less empirical information describing how the performance of an intermediate predator varies in food webs differing in trophic complexity. In this paper, I examined how the simplification of a partial IGP food web via the removal of top predators and shared prey affects the performance of the intermediate predator, *A. opacum*. Examining how the performance of *A. opacum* varies in

food webs differing in trophic complexity is particularly important given the keystone effect *A. opacum* can have on assemblages of larval anurans (Morin 1995, Chalcraft and Resetarits 2003).

Several lines of evidence suggest that the performance of intermediate predators should vary in food webs differing in trophic complexity. First, empirical (Dinter 2002, Eubanks and Denno 2000, Onzo et al. 2005) and theoretical (Abrams and Matsuda 1996, van Baalen et al. 2001) studies indicate that the growth and survival of predators in a simple food web (primary prey and the predator) should be less than that observed in food webs where the predator has access to alternative prey. Second, early theoretical work (Holt and Polis 1997, Polis and Holt 1992) on IGP demonstrates that the addition of top predators to a simple food web could cause the extinction of intermediate predators via predation and competition. Third, recent theoretical work (Daugherty et al. 2007, Holt and Huxel 2007) suggests that the addition of prey not shared between top and intermediate predators can alter the ability of intermediate predators to persist in the food web. Specifically, supplements of prey available exclusively to the top predators will lead to an increase in top predator population size, enhancing the intensity of intraguild interactions and, over the long term, driving the intermediate predator extinct (Daugherty et al. 2007, Holt and Huxel 2007). Over the short term, however, supplements to top predators may satiate top predators and reduce consumption rates of top predators on intermediate predators (Abrams and Matsuda 1996). Fourth, a number of studies have demonstrated that the strength of interaction between two species (e.g., a predator and prey) can depend on the presence or absence of a third species (e.g., another species of predator) (Relyea 2003, Sih et al. 1998). Although these lines of evidence suggest that the ability of intermediate predators to survive and grow should change across a broad gradient of food web complexity, no empirical study has

examined the growth and survival of an intermediate predator across a large portion of this gradient.

To evaluate the effect of trophic complexity on the performance of an intermediate predator (larval *Ambystoma opacum*), I conducted an experiment where I focused on disassembling (or simplifying) one of the more trophically complex food webs in which I have encountered larval *Ambystoma opacum* in nature. The most trophically complex food web is best described as partial IGP (Figure 2a). I disassembled a partial IGP food web by independently removing the top predator and a prey species shared by top and intermediate predators. These manipulations produced three simplified food webs that I refer to as the "predation" food web (Fig. 2b), the "shared prey" food web (Fig. 2c), and the "simple" food web (Fig. 2d).

Because I focused on the response of a particular life history stage (larval) of an intermediate predator, I cannot explicitly test predictions of IGP theory (Briggs and Borer 2005). IGP theory revolves around equilibrium dynamics and studies explicitly testing theory should utilize organisms with short generation times. Nonetheless, prior work has demonstrated that the survival and growth of individuals during the larval stage of the intermediate predator (*A. opacum*) that I studied can play an important role in adult demography (Scott 1994) and population regulation (Taylor and Scott 1997, Taylor et al. 2006). I expect that the simplification of a food web by removing shared prey will result in lower growth and survival of *A. opacum* due to lower resource availability. I also expect that simplifying a food web by removing top predators will enhance intermediate predator survival but the effect on growth will depend on the relative importance of a variety of different mechanisms. For example, the loss of top predators could: 1.) decrease intermediate predator growth if the presence of top predators scares

intermediate predators into foraging less (Crumrine and Crowley 2003, Volker and Armstrong 2008), 2.) increase intermediate predator growth if the presence of top predators alleviates intraspecific competition among intermediate predators by thinning the population size of intermediate predators and/or iii.) enhance intermediate predator growth if top predators are important interspecific competitors with intermediate predators. Although I expect the loss of top predators and shared prey to affect intermediate predator growth in an additive fashion, I expected that their combined loss would affect intermediate predator survival in a non-additive way. I expected a non-additive effect on intermediate predator survival because shared prey presence could detract top predators from consuming as many intermediate predators.

The effect of food web complexity on intermediate predator performance could depend on the abundance of prey present. For example, the effect of eliminating top predators from a food web may be stronger in food webs with a high abundance of prey if prey abundance is sufficient to support larger populations of predators (Finke and Denno 2005). In the absence of prey supporting larger top predator populations, I expect food web simplification to affect intermediate performance to a greater extent when few prey are present. The impacts of top predators on intermediate survival and growth will be weaker because other prey are available to be consumed by top predators. I also expect the impacts of shared prey on intermediate predator survival and growth will be weaker as the total abundance of prey increases. Consequently, I evaluated the effects of food web simplification on intermediate predator performance in partial IGP food webs that had either a low or high abundance of total (shared and unshared) prey available.

Methods

Study system

Larvae of *Ambystoma opacum* are common in ephemeral ponds in the eastern US and can function as keystone predators of larval anurans (Morin 1995, Chalcraft and Resetarits 2003). *A. opacum* breeds in the fall and lays eggs in portions of fishless ponds that are dry during the fall and typically metamorphose late in the spring. A breeding pond for *A. opacum* does not need to be completely dry during oviposition (Petranka 1998) and I have found *A. opacum* larvae in ponds in eastern NC and southeastern VA that contain larvae of other species (e.g., dragonfly naiads and Ranid frogs) that were oviposited into the pond prior to the fall. When present, larval aeschnid dragonflies (primarily *Anax* spp.) fill the top-predator trophic position in a pond thus they can consume larval *A. opacum*. Both *Anax* and *A. opacum* consume macroinvertebrates and small larval anurans during the spring (e.g., *Pseudacris*), but *A. opacum* are unable to eat some of the larger prey (e.g., overwintered *Rana* spp. tadpoles) that *Anax* can eat (Van Buskirk 1988, Wilbur and Fauth 1990, Morin 1995, Chalcraft and Resetarits 2003). Feeding trials indicate that larval *A. opacum* eat zooplankton (primarily *Daphnia* spp. and copepods) in the fall while later instar *Anax* do not (Davenport, unpublished data). Throughout their larval period, larval *A. opacum* and overwintered *Rana* tadpoles are both equally susceptible to *Anax* since neither species reaches a size refuge to avoid consumption by *Anax* (Relyea and Yurewicz 2002, Davenport, pers. obs.). In eastern NC, the most trophically complex food web in which I have found larval *A. opacum* included *Anax*, spring deposited tadpoles (primarily *P. crucifer*), overwintered tadpoles (primarily *Rana sphenocephala*) and zooplankton. I have also encountered *A. opacum* in natural ponds representing the simplified food webs in this study. I focus on growth (mass at metamorphosis) and survival of larval *A. opacum* because these

characteristics have been found to play an important role in adult demography (Scott 1994) and population regulation (Taylor and Scott 1997, Taylor, et al. 2006) of *A. opacum* populations.

Experimental Design

I assessed growth and survival of 12 *A. opacum* embedded within a partial IGP food web, and in three simpler food webs that arise from the independent removal of top predators (2 *Anax* spp. individuals) and shared prey (*Pseudacris crucifer*) (Fig. 2). These manipulations produced three food webs that were trophically simpler than the partial IGP food web in the sense that they contained fewer species or fewer trophic links. The predation food web and shared prey food web have the same number of species present but the number of trophic links is greater in the predation food web. I simplified two partial IGP food webs that differed in total prey abundance. These manipulations produce a total of eight treatments; four treatments differing in food web complexity with a low abundance of total prey and four treatments differing in food web complexity with a high abundance of total prey (Table 1).

My experiment was conducted in mesocosms, modified 1100 L cattle tanks designed to mimic natural ponds in eastern NC (Morin 1983, Wilbur 1997, Resetarits and Fauth 1998). Mesocosms represent an important venue to study the ecology of larval amphibians because they allow the experimenter to create many identical and independent experimental units to which they can apply particular treatments of interest (Morin 1989, Wilbur 1989). Although there is some disagreement about the utility of mesocosm studies (Jaeger and Walls 1989, Morin 1989, Wilbur 1989, Skelly 2002, Chalcraft et al. 2005), studies conducted in natural ponds have identified that the same processes found to be important to the ecology of larval amphibians in

mesocosms are also important in natural ponds (e.g., Petranka 1989, Scott 1990, Resetarits and Fauth 1998, Rubbo et al. 2006).

I arranged 32 mesocosms into four spatial blocks of eight at the West Research Campus of East Carolina University and performed all field procedures described below on a block by block basis. All mesocosms were filled with well water on 31 January -2 February 2007 and each received 1 kg of hardwood leaf litter on 9 February 2007 to provide a natural refuge and nutrient source for the pond food web. Mesocosms were equipped with standpipes and screen covers to allow water overflow during rain events without the loss of study organisms. Furthermore, mesocosms were covered with a fiberglass mesh lid to contain experimental animals and to prevent the colonization of non-experimental organisms.

Each of the eight treatments was randomly assigned to one mesocosm within each of the four blocks. Abundances of organisms added to a mesocosm assigned to a particular treatment (Table 1) are within the range of abundances observed within natural pond communities (Petranka 1989, Morin 1995, Relyea 2000). Inoculations of zooplankton to mesocosms were initially made on 8 February 2007 and then repeated every month for the remainder of the experiment. Inoculations were obtained by concentrating several sweeps of a fine mesh net in a natural pond into a bucket of water. Large invertebrates were removed from the inoculations as they could function as additional predators or prey resources. The majority of the zooplankton present in a 4 Liter sample obtained from each mesocosm near the end of the experiment (24 June 2007) were cladocerans. The average abundance of zooplankton in mesocosms designated to have a low abundance of prey (mean ± SE = 58.29 $\pm$ 5.09 individuals/L) was half the abundance observed in mesocosms designated to have a high abundance of prey (mean ± SE = 108.42 $\pm$ 8.81 individuals/L). *Anax*, overwintered *Rana sphenocephala* tadpoles, and *A. opacum*

were collected from the Croatan National Forest on 12-22 February 2007 and randomly assigned to the appropriate (based on treatment assigned to the mesocosm) mesocosms on 19-24 February 2007. Larval *A. opacum* added to mesocosms had an mean mass of 4.27 g (SE $\pm$ 0.92 g) and all individuals were similar in body size. Larval *Anax* introduced into mesocosms were final instar stages with a head width range from 5.2-9.1 mm. Newly hatched *P. crucifer* were collected from amplexing pairs of adults on 20 February 2007 and added to mesocosms on 2 March 2007. The densities of shared prey (Table 1) in this study are well within the realm of densities that *A. opacum* experiences in nature (Fauth and Resetarits 1991, Morin 1995).

Mesocosms were monitored daily and metamorphosed salamanders (individuals with complete absorption of the gills) and frogs (defined by emergence of at least one forelimb) were captured and returned to the lab where I recorded wet mass (g) and date of collection of each individual. *A. opacum* survival was measured as the log of the proportion of individuals (+ 0.01) that survived to metamorphosis to provide a measure of instantaneous per capita mortality rates. I did not include *A. opacum* larval period as a response variable because I collected larvae after hatching and could not accurately determine hatching dates. The size of larval dragonflies increased by the end of the experiment but growth rates did not differ among treatments with *Anax* present. Seven larval dragonflies metamorphosed prior to the end of the experiment but timing of dragonfly metamorphosis was not associated with treatment. Metamorphosed dragonflies were replaced within 24 hr by a larval *Anax* that was comparable in size to the larval *Anax* that had just metamorphosed. All mesocosms were drained between 26-27 June 2007 with meticulous searches of leaf litter for surviving larval amphibians and larval dragonflies. The wet mass (g) of larval amphibians and dragonfly naiads was recorded and all remaining animals were released at site of capture.

Statistical Analyses

We evaluated the effect of food web complexity on *A. opacum* mass at metamorphosis and survival by conducting a factorial ANOVA that specified the main and interactive effects of 1) *Anax* presence/removal and 2) shared prey presence/removal. I performed a separate factorial ANOVA for treatments that had a low abundance of prey and for treatments that had a high abundance of prey because the abundances of shared prey varied when present (200 versus 600). Although the abundance of shared prey (absent versus 200 or 600) is confounded with food web complexity (simple food web versus a simple food web with shared prey) when all eight treatments are included in the ANOVA, the confounding nature of the design disappears when treatments are analyzed in the way outlined here. Logistical constraints prevented me from using an experimental design that would have allowed us to evaluate the independent and interactive effects of top predator presence, shared prey presence, and total prey abundance. Consequently, this approach resulted in two different analyses (one for each level of prey abundance) that compared *A. opacum* performance in each of the four treatments (environments) that differed in trophic complexity (Fig. 2). Each of the main effects in the factorial ANOVA describe the effect of simplifying the partial IGP food web by removing either shared prey or top predators while the interaction term evaluates whether simplification as the result of the simultaneous removal of top predators and shared prey results in a change in *A. opacum* that is different from what would be expected if the removal of top predators and shared prey affect *A. opacum* independently of each other.

Although confounding factors prevent me from evaluating the interactive effects of shared prey removal from partial IGP food webs and the abundance of unshared prey present in

the food web, I can evaluate the interactive effects of top predator removal and unshared prey abundance by focusing on the loss of predators from food webs lacking shared prey. I evaluated the independent and interactive effects of top predator removal and the abundance of unshared prey in food webs lacking shared prey with a factorial ANOVA. The exclusion of food webs with shared prey in this analysis restricts the scope of inference of these results to a narrower range of environments differing in food web complexity (i.e., a simple food web and the predation food web versus the four different food webs described in Fig. 2) but it does provide some insight into how the loss of top predators affects intermediate predators in food webs that differ in prey abundance.

I analyzed the survival unshared prey, *Rana*, with a factorial ANOVA that included the factors *Anax* presence/absence, *P. crucifer* presence/absence, and *Rana* density. The ANOVA also included all two way and three way interactions between the factors. *P. crucifer* response variables were unable to be statistically compared between the two food webs (the complex food web and the intermediate complexity food web that did not contain *Anax*) in which *P. crucifer* was present due to the fact that no *P. crucifer* survived in any replicate ponds lacking *Anax*. Residuals for all data were visually inspected for normality to meet ANOVA assumptions. Block effects (and all interactions involving block effects) were originally included in all analyses. These results, however, are not presented here for simplicity and their inclusion does not alter the interpretation of results as presented here.

Results

Decreasing food web complexity via the removal of *Anax* from partial IGP food webs with a low abundance of unshared prey caused an increase in *A. opacum* survival ($F_{1, 12} = 21.38$,

$P < 0.001$; Fig. 3) and did not alter *A. opacum* mass at metamorphosis ($F_{1, 7} = 4.13$, $P = 0.088$; Fig. 3). In contrast, decreasing food web complexity via the removal of shared prey from partial IGP food webs with a low abundance of prey did not affect survival ($F_{1, 12} = 1.09$, $P = 0.317$; Fig. 3) or mass at metamorphosis of *A. opacum* ($F_{1, 7} = 1.49$, $P = 0.261$; Fig. 3). The interaction between the effect of *Anax* removal and the effect of shared prey removal did not influence either *A. opacum* survival ($F_{1, 12} = 0.04$, $P = 0.843$; Fig. 3) or mass at metamorphosis ($F_{1, 7} = 0.16$, $P = 0.701$; Fig. 3) in food webs with a low abundance of unshared prey.

Similarly, the removal of *Anax* from partial IGP food webs with a high abundance of prey caused an increase in *A. opacum* survival ($F_{1, 12} = 50.78$, $P < 0.001$; Fig. 4) but greatly reduced *A. opacum* mass at metamorphosis ($F_{1, 8} = 55.72$, $P < 0.001$; Fig. 4). Removal of shared prey from partial IGP food webs with a high abundance of unshared prey did not affect *A. opacum* survival ($F_{1, 12} = 1.74$, $P = 0.212$; Fig. 4), but did decrease *A. opacum* mass at metamorphosis ($F_{1, 8} = 13.02$, $P = 0.007$; Fig. 4). The interaction between the effect of *Anax* removal and the effect of shared prey removal did not influence *A. opacum* survival ($F_{1, 12} = 1.44$, $P = 0.253$; Fig. 4) in food webs with a high abundance of prey but the interaction did influence *A. opacum* mass at metamorphosis ($F_{1, 8} = 9.74$, $P = 0.014$; Fig. 4). This significant interaction indicates that the effect of the combined removal of *Anax* and shared prey on *A. opacum* mass at metamorphosis is different from what would be expected given observed responses of *A. opacum* to the independent removal of *Anax* and shared prey from the partial IGP food web. Specifically, *A. opacum* metamorphose at approximately the same size when both *Anax* and shared prey are removed as when *Anax* alone is removed from the partial IGP food web, even though the removal of shared prey alone from the partial IGP food web also causes *A. opacum* to metamorphose at smaller sizes. Consequently, it appears that the effect of shared prey removal

on *A. opacum* mass at metamorphosis is completely subsumed within the effect of *Anax* removal when both species are removed simultaneously.

Increasing the abundance of unshared prey in simple food webs lacking shared prey did not alter *A. opacum* survivorship ($F_{1, 12} = 0.37$, $P = 0.556$; Fig. 5) or *A. opacum* mass at metamorphosis ($F_{1, 8} = 1.36$, $P = 0.278$; Fig. 5). *Anax* removal from predation food webs resulted in an increase in *A. opacum* survivorship ($F_{1, 12} = 34.53$, $P < 0.001$; Fig. 5) and a reduction in *A. opacum* mass at metamorphosis ($F_{1, 8} = 8.33$, $P = 0.02$; Fig. 5). The abundance of unshared prey did not alter the effect of *Anax* removal on either *A. opacum* mass at metamorphosis ($F_{1, 8} = 1.34$, $P = 0.280$; Fig. 5) or *A. opacum* survival ($F_{1, 12} = 0.27$, $P = 0.610$; Fig. 5).

Survival of larval anurans (unshared and shared prey) differed among the different food webs. The effect of *Anax* on *Rana* survival depended on the density of *Rana* present (statistical interaction between presence of *Anax* and *Rana* density: $F_{1, 24} = 6.33$, $P = 0.019$). Specifically, *Anax* reduced *Rana* survival when *Rana* density was high but enhanced *Rana* survival when *Rana* density was low (Appendix A). Independent of the synergistic effect between *Anax* presence and *Rana* density, there was a strong trend for *Rana* survival to increase as *Rana* density increased ($F_{1, 24} = 3.36$, $P = 0.079$; Appendix A). Neither the removal of *P. crucifer*, nor any statistical interactions involving *P. crucifer* removal accounted for a significant amount of variation in *Rana* survival (all $F_{1, 24} \leq 0.47$, $P \geq 0.500$). *P. crucifer*, the shared prey, only survived to metamorphosis in the partial IGP food web (mean proportion surviving $\pm$ 95% CI in food webs with low prey abundance = 0.05 ± 0.06; mean proportion surviving $\pm$ 95% CI in food webs with high prey abundance = 0.02 ± 0.03).

Discussion

I found that simplifying a partial IGP food web via the removal of top predators and shared prey can have detrimental effects on growth and survival of the intermediate predator, *A. opacum*. Obviously, an *A. opacum* individual that does not survive the larval environment will have no reproductive success. For those ambystomatid larvae that do survive, individuals with higher mass at metamorphosis experience an earlier age at first reproduction, larger size at first reproduction, increased fecundity (larger clutch size for females) and a greater chance of surviving to their first reproductive event (Semlitsch et al. 1988, Scott 1994). Simulation models incorporating data from natural populations of *A. opacum* indicate that variation in larval survival and mass at metamorphosis can have important consequences for the long-term dynamics of *A. opacum* populations (Taylor and Scott 1997, Taylor et al. 2006). Both of these life history responses clearly have significant consequences for the overall fitness of individual *A. opacum* and persistence of *A. opacum* populations.

Although I expected to see that the loss of top predators from a food web would enhance intermediate predator survival, the loss of shared prey did not affect either intermediate predator survival or the effect of top predators on intermediate predator survival. These observations reinforce the idea that top predators play a particularly important role in pond food webs (McPeek 1998, Chalcraft and Resetarits 2003). The results also suggest that competition for prey was not severe enough to cause intermediate predators to die. The fact that the loss of shared prey did not alter the impact of top predators on intermediate predator survival also suggests that alternative prey did not satiate predators as originally predicted. Although it is possible that a higher abundance of prey would have satiated top predators, the abundances of prey that I used are reflective of prey abundances found in nature. In this case, the loss of a top

predator from a partial IGP food web has the same effect on intermediate predator survival as the loss of a top predator from a traditional IGP food web. This important finding indicates that the effect of shared and alternative prey on intermediate predator survival may only manifest in longer term studies if alternative prey support larger populations of top predators that exert more negative effects on intermediate predator survival (Daugherty et al. 2007, Holt and Huxel 2007).

One of the most interesting results of my study is that food web simplification affected the growth of *A. opacum* differently in food webs that differed in total prey abundance. Simplification of a partial IGP food web had no effect on *A. opacum* size at metamorphosis in food webs with a low abundance of prey but reduced *A. opacum* size at metamorphosis in food webs with a high abundance of prey. The loss of either top predators or shared prey from a partial IGP food web with a high abundance of prey caused intermediate predators to metamorphose at a smaller size but the effect of shared prey loss on intermediate predator growth was less than the effect of top predator loss. Based on the statistical analyses, the removal of top predators and shared prey from partial IGP food webs with a high abundance of total prey had a non-additive effect on salamander mass at metamorphosis. The removal of both top predators and shared prey from a partial IGP food web resulted in *A. opacum* metamorphosing at a size that was comparable to that observed when only top predators were removed from the partial IGP food web. Given the importance of size at metamorphosis to the long-term dynamics of *A. opacum* (Taylor and Scott 1997, Taylor et al. 2006), these findings indicate that food web simplification will have a greater impact on the long-term dynamics of intermediate predators when prey abundance is rather high versus low.

One potential explanation for why I failed to detect statistically significant effects of food web simplification on *A. opacum* mass at metamorphosis in food webs with a low abundance of

prey is that I lacked statistical power. Reasons for reduced statistical power include greater within treatment variability or smaller treatment effects in low abundance food webs. To ensure that the effects of food web simplification are different in food webs with a high abundance of prey than in food webs with a low abundance of prey, I evaluated the statistical power to detect treatment effects in food webs with a low abundance of prey that were as large as the treatment effects observed in food webs with a high abundance of prey. I found that the statistical power of the analyses on *A. opacum* mass at metamorphosis in food webs with a low abundance of prey was adequate (β of predator effect= 0.99, β of shared prey effect= 0.91, β of predator x shared prey interaction=0.78) to detect differences in *A. opacum* mass at metamorphosis that were as large as those observed in food webs with a high abundance of prey. Consequently, even without greater sample sizes in food webs with a low abundance of prey, I had sufficient statistical power to conclude that food web simplification affects *A. opacum* mass at metamorphosis differently in food webs varying in total prey abundance.

The simplification of partial IGP food webs had complex effects on intermediate predator growth in food webs with a high abundance of prey, indicating my initial hypothesis about an additive response to top predator and shared prey loss was incorrect. I believe that simplifying partial IGP food webs with a high abundance of prey resulted in smaller salamanders because the removal of top predators and shared prey resulted in the loss of two important processes that promote salamander growth. First, the loss of top predators from a partial IGP food web resulted in smaller salamanders, in part, because top predators benefitted surviving intermediate predators by thinning the number of intermediate predators which reduced competition for prey resources. The occurrence of thinning is reflected by the fact that salamanders metamorphosed at a larger size in the predation food web than in the simple food web (Fig. 3). The beneficial effects of

thinning in promoting the growth of prey has been reported in other studies as well (Van Buskirk and Yurewicz 1998, Relyea 2002, Relyea 2007). Second, I also found evidence that the loss of shared prey from a partial IGP food web increased competition which resulted in smaller salamanders (Fig. 3). In the absence of thinning by top predators, however, the availability of shared prey did not appear to be sufficient to reduce competition because there was no difference in salamander size in the simple food web and the shared prey food web (both of which lacked top predators). These results indicate that thinning the population size of intermediate predators augments the beneficial effects of alternative prey availability. The removal of top predators from a partial IGP food web with a high abundance of prey effectively resulted in the loss of the beneficial effects of thinning and the effects of supplemental prey availability because there was no change in salamander size in the simple food web and the shared prey food web. The simplification of a partial IGP food web with a low abundance of prey had no effect (or at least weaker effects than in food webs with a high abundance of prey) on intermediate predator growth because predator thinning and the amount of shared prey present was insufficient to ameliorate strong competition for a low amount of resources. My results indicate that the way in which a partial IGP food web is simplified can have important effects on intermediate predator growth but the loss of top predators has the most striking effect. The removal of top predators represents the loss of two processes that enhance intermediate predator growth while the removal of shared prey results in the loss of one process.

My finding that simplification of a partial IGP food web and total prey abundance interact synergistically to affect *A. opacum* performance suggests that among pond variation in food web complexity and prey abundance could generate source and sink habitats for *A. opacum* metapopulations. Source ponds (partial IGP food webs) may be contributing to the persistence

of *A. opacum* populations by providing larger, more fecund salamanders to colonize or rescue nearby sink ponds (simplified food webs in my study) that produce smaller individuals that will not survive long after metamorphosis (Petranka 1989, Scott 1990). If sink ponds (simple food webs) produce smaller individuals that do live to reproduce, then the sink populations will still likely have lower population growth rates (compared to source population growth rates) unless they gain migrants from source populations. Dispersal between ponds of various qualities is thought to be responsible for causing fluctuations in the size of amphibian populations (Semlitsch et al. 1996, Marsh and Trenham 2001, Smith and Green 2005).

My results also suggest that food web complexity may facilitate the ability of some predator species to fulfill a keystone role in ecological food webs by preventing keystone predators from becoming very abundant. *A. opacum* is a known keystone predator that enhances survival of competitively inferior larval anurans by selectively consuming competitively dominant larval anurans (Morin 1995, Chalcraft and Resetarits 2003). At high abundances, however, the beneficial effects of keystone predators on inferior prey can be diminished as inferior prey have a greater probability of being consumed (Morin 1983). I suggest that *A. opacum* is more likely to operate in a keystone manner in trophically complex food webs where top predators (such as *Anax*) reduce *A. opacum* overall abundance.

Although the short-term nature of my study (< 1 generation of the study animals) prevents me from directly testing the equilibrial conditions predicted by IGP theory, I believe that my findings and the results of others demonstrating the importance of larval survival and growth on fitness and population dynamics of *A. opacum* shed important light on how the simplification of a partial IGP food web will affect populations of *A. opacum*. My study supports the argument (Daugherty et al. 2007, Holt and Huxel 2007) that intermediate predators

are more likely to persist over a broader range of conditions with top predators when there is a sufficient abundance of alternative prey available to predators. In my case, however, alternative prey do not enhance the survival of intermediate predators by reducing the consumptive effect of top predators on intermediate predators. Instead my empirical data provides the novel contribution that in partial IGP food webs, alternative prey support intermediate predator growth rates when top predators are present by augmenting the beneficial effects of thinning by top predators on intermediate predators.

References

Abrams, P.A. and H. Matsuda. 1996. Positive indirect effects between prey species that share predators. Ecology 77:610-616.

Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71:1599-1608.

Borer, E.T. 2002. Larval competition of guild members: implications for coexistence via intraguild predation. Journal of Animal Ecology 71:957-965.

Briggs, C.J. and E.T. Borer. 2005. Why short-term experiments may not allow long- term predictions about intraguild predation. Ecological Applications 15:1111-1117.

Carey, M.P. and D.H. Wahl. 2010. Interactions of multiple predators with different foraging modes in an aquatic food web. Oecologia 162:443-452.

Chalcraft, D.R. and W.J.Resetarits, Jr. 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? Ecology 84:2407-2418.

Chalcraft, D.R., C.A. Binckley and W. J., Resetarits, Jr. 2005. Experimental venue and estimation of interaction strength: Comment. Ecology 86:1061-1067.

Crumrine, P.W. and P.H. Crowley. 2003. Partitioning components of risk reduction in a dragonfly-fish intraguild predation system. Ecology 84:1588-1597.

Daugherty, M.P., J.P. Harmon, and C.J. Briggs. 2007. Trophic supplements to intraguild predation. Oikos 116:662-677.

Dinter, A. 2002. Microcosm studies on intraguild predation between female erigonid spiders and lacewing larvae and influence of single versus multiple predators on cereal aphids. Journal of Applied Entomology 126:249-257.

Eubanks, M.D. and R.F. Denno. 2000. Host plants mediate omnivore-herbivore interactions and influence prey suppression. Ecology 81:936-947.

Fauth, J.E. and W.J. Resetarits, Jr. 1991. Interactions between the salamander *Siren intermedia* and the keystone predator *Notophthalmus viridescens*. Ecology 72: 827-838.

Finke, D. L. and R. F. Denno. 2004. Predator diversity dampens trophic cascades. Nature 429:407-410.

Finke, D.L. and R.F. Denno. 2005. Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. Ecology Letters 8:1299-1306.

Holt, R.D. and G.A. Polis. 1997. A theoretical framework for intraguild predation. American Naturalist 149:745-764.

Holt, R.D. and G.R. Huxel. 2007. Alternative prey and the dynamics of intraguild predation: theoretical perspectives. Ecology 88:2706-2712.

Jaeger, RG. And S.C. Walls. 1989. On salamander guilds and ecological methodology. Herpetologica 45:111-119.

Lannoo, M. 2005. Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, CA, USA.

Marsh, D.M. and P.C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology 15:40-49.

McPeek, M.A. 1998. The consequences of changing the top predator in a food web: a comparative experimental approach. Ecological Monographs 68:1-23.

Morin, P.J. 1983. Predation, competition, and the composition of larval anuran guilds. Ecological Monographs 53:119-138.

Morin, P.J. 1989. New directions in amphibian community ecology. Herpetologica 45:124-128.

Morin, P.J. 1995. Functional redundancy, non-additive interactions, and supply-side dynamics in experimental pond communities. Ecology 76:133-149.

Onzo, A., R. Hanna, K. Negloh, M. Toko, and M.W. Sablelis. 2005. Biological control of cassava green mite with exotic and indigenous phytoseiid predators – effects of intraguild predation and supplementary food. Biological Control 33:143-152.

Petranka, J.W. 1989. Density-dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. Ecology 70:1752-1767.

Polis, G.A. and R.D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends in Ecology and Evolution 7:151-154.

Polis, G.A., C.A.Myers, and R.D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297-330.

Relyea, R.A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. Ecology 81:2278-2289.

Relyea, R.A. 2002. The many faces of predation: How selection, induction, and thinning combine to alter prey phenotypes. Ecology 83:1953-1964.

Relyea, R.A. 2003. Predators come and go: the reversibility of predator-induced traits. Ecology 84:1840-1848.

Relyea, R.A. 2007. Getting out alive: how predators affect the decision to metamorphose. Oecologia 152:389-400.

Relyea, R.A. and K.L. Yurewicz. 2002. Predicting community outcomes from pairwise interactions: integrating density- and trait-mediated effects. Oecologia 131:569-579.

Resetarits, W.J., Jr. and J.E. Fauth. 1998. From cattle tanks to Carolina bays: the utility of model systems for understanding natural communities. Pages 133-151 *in* W.J. Resetarists, Jr and J. Bernardo, editors. Experimental Ecology: Issues and Perspectives. Oxford University Press, New York, USA.

Rubbo, M.J., K. Shea, and J.M. Kiesecker. 2006. The influence of multi-stage predation on population growth and the distribution of the pond-breeding salamander, *Ambystoma jeffersonianum*. Canadian Journal of Zoology 84:449-458.

Rudolf, V.H.W. and J. Armstrong. 2008. Emergent impacts of cannibalism and size refuges in prey on intraguild predation systems. Oecologia 157:675-686.

Scott, D.E. 1990. Effects of larval density in *Ambystoma opacum*: an experiment in large scale field enclosures. Ecology 71:296-306.

Scott, D.E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology 75:1383-1396.

Semlitsch, R.D., D.E. Scott, and J.H.K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184-192.

Semlitsch, R.D., D.E. Scott, J.H.K. Pechmann, and J.W. Gibbons. 1996. Structure and dynamics of an amphibian community: evidence from a 16-year study of a natural pond. Pages 217-248 *in* M.L. Cody and J.A. Smallwood, editors. Long-Term Studies of Vertebrate Communities. Academic Press, San Diego, CA, USA.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology and Evolution 13:350-355.

Skelly, D.K. 2002. Experimental venue and estimation of interaction strength. Ecology 83:2097-2101.

Smith, M.A. and D.M. Green. 2005. Are all amphibian populations metapopulations? Dispersal and the metapopulation paradigm in amphibian ecology. Ecography 28:110-128.

Taylor, B.E., and D.E. Scott. 1997. Effects of larval density dependence on population dynamics of *Ambystoma opacum*. Herpetologica 53:132-145.

Taylor, B.E., D.E. Scott, and J.W. Gibbons. 2006. Catastrophic reproductive failure, terrestrial survival, and persistence of the marbled salamander. Conservation Biology 20:1457-1465.

Thompson, R.M., M. Hemberg, B.M. Starzomski, and J.B. Shurin. 2007. Trophic levels and trophic tangles: the prevalence of omnivory in real food webs. Ecology 88:612-617.

Van Baalen, M., V. Krivan, P.C.J. van Rijn, and M.W. Sabelis. 2001. Alternative food, switching predators, and the persistence of predator-prey systems. American Naturalist 157:512-524.

Van Buskirk, J. 1988. Interactive effects of dragonfly predation in experimental pond communities. Ecology 69:857-867.

Van Buskirk, J. and K.L. Yurewicz. 1998. Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. Oikos 82:20-28.

Wilbur, H.M. 1989. In defense of tanks. Herpetologica 45:122-123.

Wilbur, H.M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology 78:2279-2302.

Wilbur, H.M. and J.E. Fauth. 1990. Experimental aquatic food webs: interactions between two predators and two prey. American Naturalist 135:176-204.

Yurewicz, K. L. 2004. A growth/mortality trade-off in larval salamanders and the coexistence of intraguild predators and prey. Oecologia 138:102-111.

Table 1. Abundances of organisms present in each of the eight food web treatments considered in my study. All abundances represent number of individuals/mesocosm except for zooplankton. Abundance for zooplankton represents volume of inoculum.

		Top Predator	Intermediate Predator	Shared Prey	Prey not shared by *Anax*	Prey not shared by *A. opacum*
Treatment	Prey abundance	*Anax*	*A. opacum*	*P. crucifer*	*Rana*	Zooplankton
Simple	Low	0	12	0	5	450 mL
Predation		2	12	0	5	450 mL
Shared		0	12	200	5	450 mL
Partial IGP		2	12	200	5	450 mL
Simple	High	0	12	0	15	1350 mL
Predation		2	12	0	15	1350 mL
Shared		0	12	600	15	1350 mL
Partial IGP		2	12	600	15	1350 mL

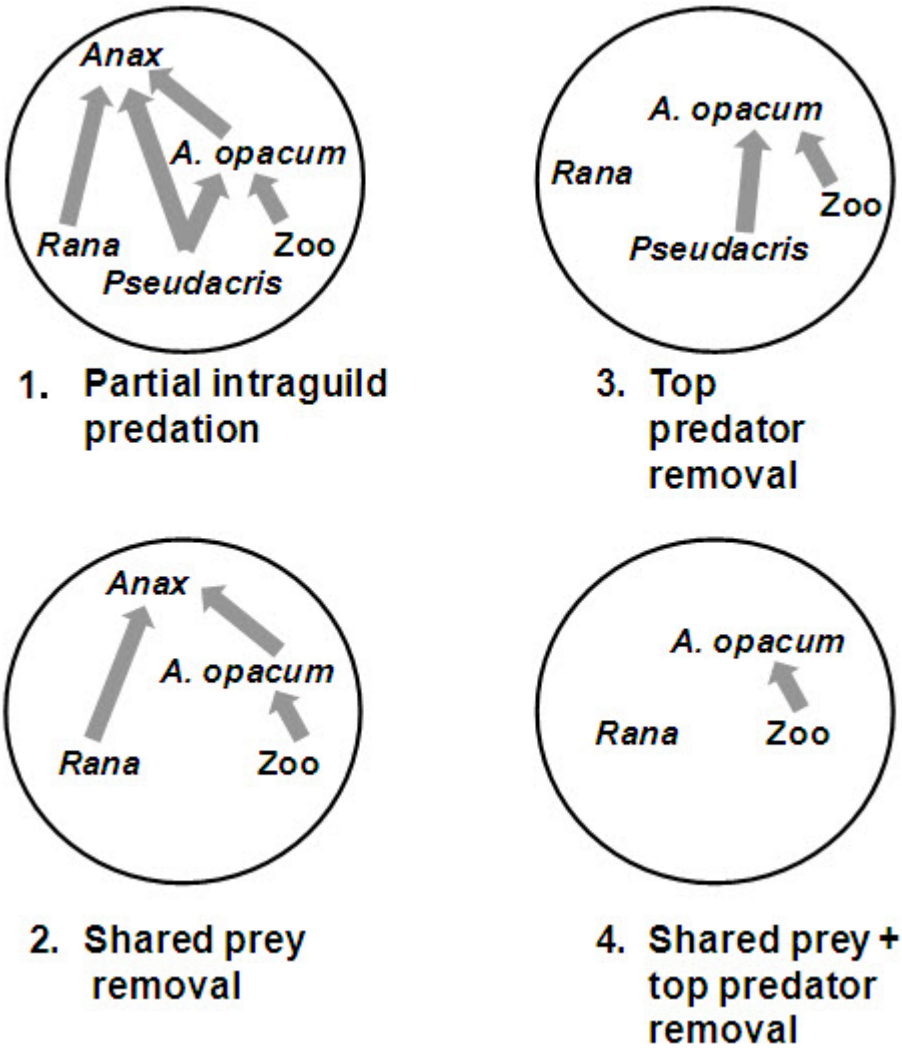


Figure 2. Examples of natural food webs in which *Ambystoma opacum* can be found in eastern North Carolina. In each food web, the following letters stand for, *Rana* = Overwintered, large *Rana* tadpoles, Zoo = Zooplankton, and *Pseudacris* = Spring peeper tadpoles, *P. crucifer*. Each circle represents a food web in my study with solid arrows describing the feeding relationships within each food web. Trophic complexity, in terms of the number of species and trophic links present, decreases as *Anax* and shared prey are removed from the partial IGP food web. Although simpler than the partial IGP food web (1), the predation food web (2) is more trophically complex than the shared prey food web (3) because it has more trophic links.

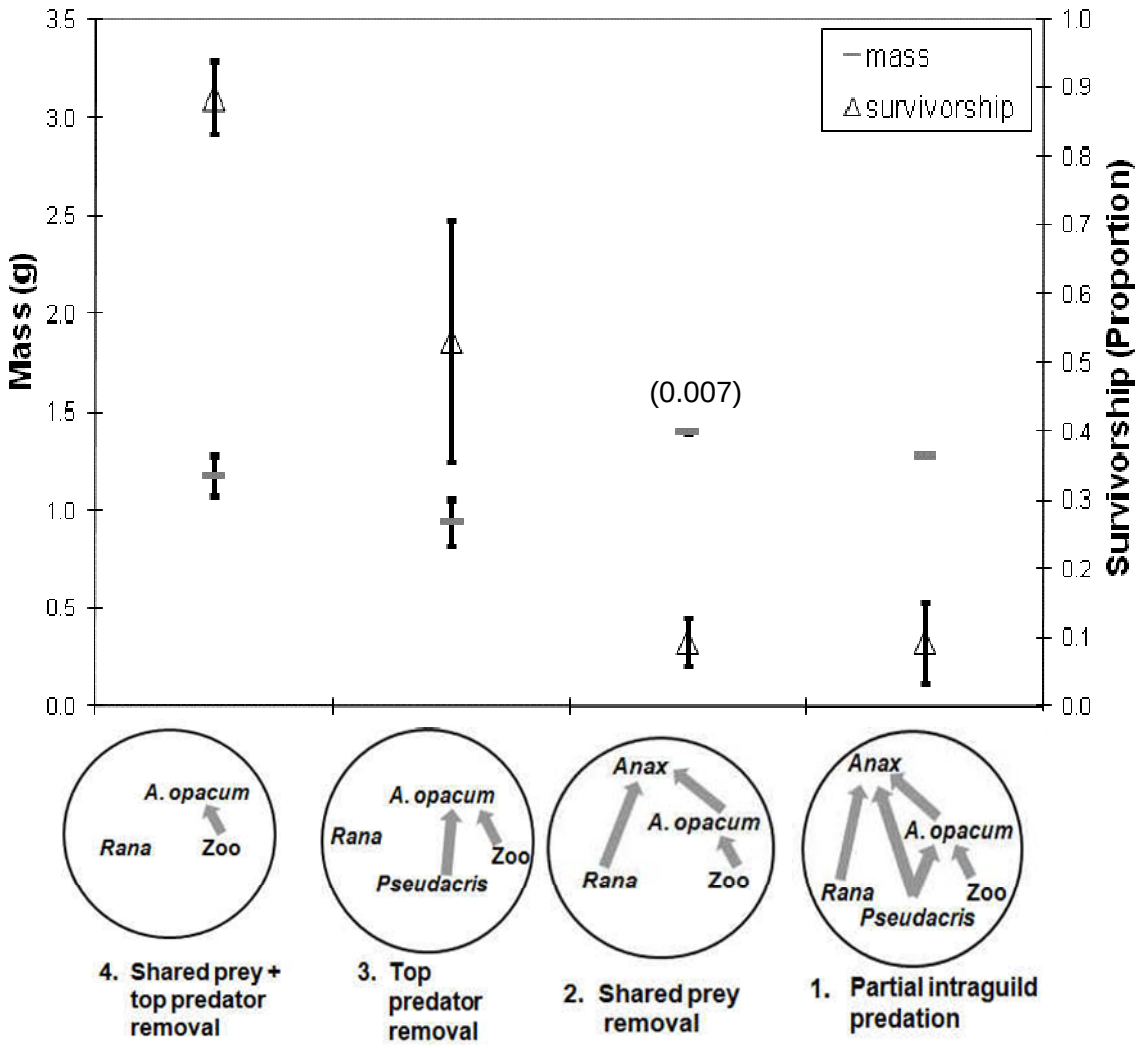


Figure 3. Mean (+ 1 SE) survivorship and mass at metamorphosis of A. opacum in food webs of varying complexity with a low abundance of prey. Means and standard errors are based on values (i.e., either total survival or mean mass at metamorphosis of individuals within a pond) associated with an individual pond of a particular treatment. Numbers within parentheses represent the SE for a response variable in treatments where the graphical depiction of the SE is smaller than the symbol size for the average response. Samples sizes are N=4 in all cases except for mean mass at metamorphosis in the predation treatment (N=2) and the partial IGP food web treatment with low prey abundance (N=1). Sample sizes for these treatments were smaller because fewer ponds within these treatments produced surviving metamorphs.

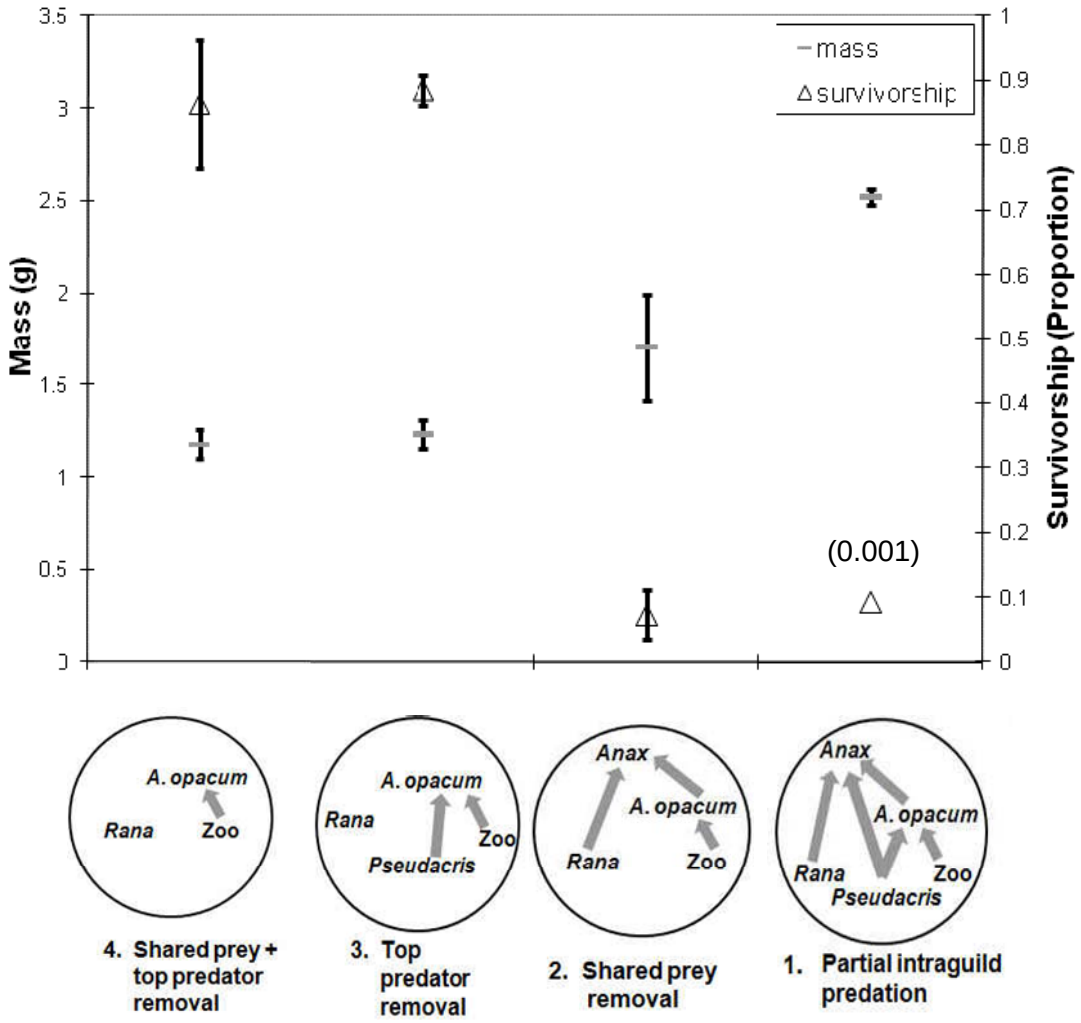


Figure 4. Mean (± 1 SE) survivorship and mass at metamorphosis of *A. opacum* in food webs of varying complexity with a high abundance of prey. Means and standard errors are based on values (i.e., either total survival or mean mass at metamorphosis of individuals within a pond) associated with an individual pond of a particular treatment. Numbers within parentheses represent the SE for a response variable in treatments where the graphical depiction of the SE is smaller than the symbol size for the average response. Samples sizes are N=4 in all cases except for mean mass at metamorphosis in the partial IGP food web treatment with high prey abundance (N=2). Sample sizes for these treatments were smaller because fewer ponds within these treatments produced surviving metamorphs.

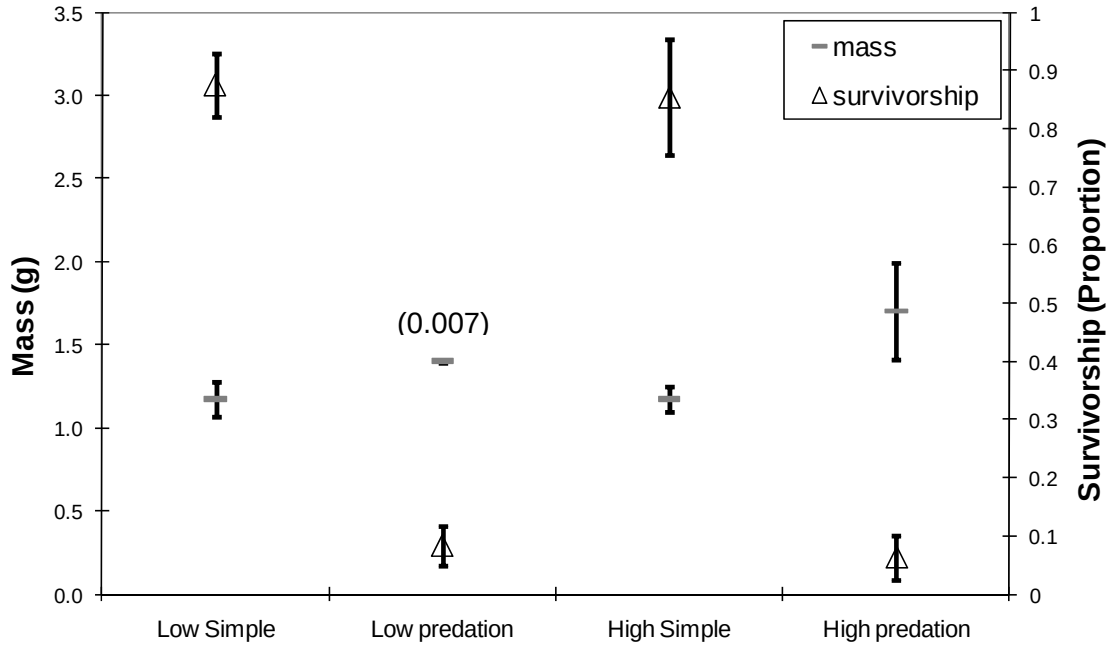


Figure 5. Mean (± 1 SE) survivorship and mass at metamorphosis of *A. opacum* in food webs lacking shared prey but varying in unshared prey abundance (low versus high) and trophic complexity (*Anax* present versus absent). Means and standard errors are based on values (i.e., either total survival or mean mass at metamorphosis of individuals within a pond) associated with an individual pond of a particular treatment. Numbers within parentheses represent the SE for a response variable in treatments where the graphical depiction of the SE is smaller than the symbol size for the average response. Samples sizes are N=4 in all cases except for mean mass at metamorphosis in treatments representing a predation food web with a low abundance of unshared prey (N=2). Sample sizes for these treatments were smaller because fewer ponds within these treatments produced surviving metamorphs.

CHAPTER 3: Larval Dragonflies Scare the Shape Out of Larval Salamanders: Trait Variation of an Intermediate Predator

Introduction

Interactions among species can play an important role in controlling the distribution and abundance of species. One type of interaction within a food web is intraguild predation (IGP); where at least two predator species compete for the same food resource (shared prey) and one predator species (top predator) can consume the other predator species (intermediate predator) (Holt and Polis 1997). Although top predators should prevent intermediate predators from coexisting with them by reducing the abundance of intermediate predators via competition and predation, intraguild predation seems to be quite common across many taxa in nature (Polis et al. 1989; Arim and Marquet 2004). One factor that may facilitate the persistence of top and intermediate predators is the induction of defenses by intermediate predators.

Many studies have documented that prey change their phenotype (morphology and behavior) in response to the presence of predators (Tollrian and Harvell 1999). Prey often reduce activity or increase use of refuges in the presence of predators (Lima and Dill 1990). Prey may also develop a morphology and/or color when exposed to predators, not exhibited by prey not exposed to predators, to decrease the likelihood of a fatal attack by a predator that prey not exposed to predators do not exhibit (Van Buskirk and Schmidt 2000). Most studies examining color and morphological changes in prey to predators have focused on predator-herbivore interactions and few have quantified trait alterations in intermediate predators to top predators (e.g., Van Buskirk and Schmidt 2000, Yurewicz 2004). Intermediate predators, like herbivores, that alter their phenotype in response to predators should experience greater fitness versus intermediate predators that do not possess a predator-induced phenotype when predators

are present (Van Buskirk 2000). The benefit of a predator-inducted phenotype may increase the fitness of an individual, but costs associated with predator-induced changes may lead to lower overall population growth rates. This could be due to reduced individual reproductive output because energy has been allocated away from reproductive tissues to defensive tissues (Lima 1998, Preisser et al. 2005, Pangle et al. 2007).

Theory suggests that predator-induced responses by prey should stabilize population and community dynamics, yet empirical work in support of theory is lacking (Abrams 2008, Mougi and Rishida 2009, Boeing and Ramcharan 2010). Recently, however, Boeing and Ramcharan (2010) provided evidence that populations of predator-induced *Daphnia pulex* were less likely to experience boom-or-bust population dynamics than populations of non-predator-induced *D. pulex* because predator- induced populations were less likely to overexploit their food resources. Furthermore, populations of predator-induced *D. pulex* were more likely to persist with fish predators than populations of non-predator-induced *D. pulex* because predator-induced phenotypes could seek refuge in the water column with low clarity due to the high growth of algal resources. Non-predator-induced phenotypes depleted algal resources and then were easily seen and consumed by fish predators since water clarity was high. Thus, the nonlethal effects of predators on prey may contribute a large role in stabilizing population dynamics and permitting the persistence of prey populations with predators (Boeing and Ramcharan 2010).

Recently, ecologists have found that organisms also have the ability to alter their phenotype in response to the presence of competitors in order to reduce mortality from competition (Relyea 2002, Teplitsky and Laurila 2007, Ashton et al. 2010). One such group of organisms, herbivorous tadpoles, expresses a competitor-induced phenotypic change that is strikingly different than a predator-induced change. Predator-induced tadpoles have deeper and

longer tails, shorter bodies and reduced activity levels while competitor-induced tadpoles have shallower and shorter tails, longer bodies and increased activity levels. With predator-induced phenotypes, a larger tail likely provides prey with a non fatal target for predator strikes and allows quicker swimming bursts to escape predators (Van Buskirk and McCollum 2000a,b). Competitor-induced phenotypes have longer bodies presumably to promote digestive efficiency when exploiting resources (Relyea 2002, Relyea and Auld 2004). Therefore evidence suggests that organisms have the ability to assess their environment and adjust traits or "fine tune" phenotypes accordingly (Benard 2004, Miner et al. 2005, Berg and Ellers 2010).

Sensitivity to environmental cues from competitors and predators is important since most organisms in ecological communities are exposed to competitive and consumptive pressure simultaneously. For example, as conspecific density increases, perceived predation risk is lowered with predators that have a saturating functional response because there are lots of other prey resources that predators could eat (Abrams 1990, Abrams et al. 1990). Thus, an energetic trade-off has been hypothesized between competitor- and predator-induced phenotypes since individuals must decide how to allocate energy toward the production of different kinds of tissues during development (e.g., produce longer bodies and short tails or produce shorter bodies and long tails)(Sih et al. 1998, Relyea and Auld 2004). Consequently, research with herbivorous tadpoles (Relyea 2002, McCoy 2007, Van Buskirk et al. in press) indicates that prey may experience this trade-off when expressing unique phenotypes dependent on the predators and competitors present in the environment. Therefore, the magnitude of phenotypic investment in response to predators may be mediated by predator conspecific density (Peacor 2003, McCoy 2007, Van Buskirk et al. in press).

One other issue that remains unclear from prior studies examining morphological changes in prey to predators or competitors is the timing during development that these trait changes occur (McCollum and Van Buskirk 1996, Relyea 2003, 2007, Hoverman and Relyea 2007). Ecologists are aware of this paucity in empirical work and have advocated more studies that integrate measurements of multiple traits during multiple developmental stages in different environments (Pigliucci 2003, West-Eberhard 2003, Boege and Marquis 2005) since expression of a particular phenotype can be affected in multiple ways. For example, at a low density of competitors, an intermediate predator might express a phenotype in response to predators that would be maintained throughout the presence of the top predator. The maintenance costs for a particular phenotype in that environment may be high, but the reward for investment is also very high with predation risk reduced greatly in the presence of predators (Schlichting and Pigliucci 1998). At a high density of competitors, however, an intermediate predator may focus on investing in a phenotype that is efficient at exploiting resources and less effective at reducing predation risk. In this example, the immediate threat to an individual is resource competition and therefore it would be more beneficial for intermediate predators to invest in traits that improve the capability to acquire resources.

In one of the few studies to address predator-induced traits throughout development, Relyea (2003) found that herbivores (larval anurans) changed their phenotypic strategy over ontogeny. Specifically, larval anurans relied primarily on anti-predator behaviors (hiding and reduced activity) during early stages of development but relied predominantly on morphological defenses during later stages of development (Relyea 2003). This work supports theoretical predictions that selection will favor the induction of alternative phenotypes as individuals track changes in the environment (Gabriel 1999, Gabriel et al. 2005). The explanation for the finding

by Relyea (2003) is that it may take a longer time for prey to alter their morphology enough to effectively reduce their risk of predation. No studies have documented how predators and competitors influence development of morphology throughout ontogeny. Such observations are important for multiple reasons. First, the induction of a particular morphology early in development may preclude other types of changes later in development. As previously mentioned, competitors could promote one type of morphology early on during development and predators could induce a different type of morphology later in development as competitor densities are reduced due to predation. Morphological responses to competitors early on in development, however could also limit future morphological changes in response to predators. Second, identifying the timing in which predators and competitors induce trait changes in intermediate predators could indicate what is assessed as the greater risk to mortality at that particular developmental stage (e.g. whether it is competitive pressure or consumptive pressure). No study has quantified how the exposure to multiple levels of conspecific density and predation cause changes in the traits of intermediate predators through ontogeny.

I tested several predictions regarding 1) whether intermediate predators can respond morphologically and behaviorally to environmental variation in top predator presence and the density of conspecifics and 2) how the responses of intermediate predators to their environment affect certain aspects of intermediate predator performance. First, I hypothesize that intermediate predators will have the ability to modify their traits in accordance to the perceived predation risk and the intensity of competitive interactions in the environment (i.e., changes in behavior, body and tail traits reflect environmental conditions perceived by intermediate predators). I predict intermediate predators will respond with a phenotype that corresponds to the greatest perceived mortality risk in the environment, either due to predation or due to

resource competition. Specifically, intermediate predators in environments with predators but few competitors will have extreme predator-induced phenotypes since perceived predation risk will be higher while intermediate predators in environments with predators and lots of competitors will have extreme competitor-induced phenotypes since perceived exploitative competition risk is higher. In environments with intermediate levels of competition and predation, I expect a phenotype that is intermediate between predator- and competitor-induced phenotypes, since it is likely that predators and competitors induce traits to change in opposite directions. Second, I predict that the timing at which predator- and competitor-induced traits are produced will be different for behavioral traits and morphological traits. Intermediate predators may not induce morphological responses to predators or competitors until later in ontogeny due to the time needed to allocate tissue for defenses, therefore behavioral traits (will be induced earlier on in ontogeny to compensate. Third, I hypothesize that the performance of intermediate predators with extreme predator-induced phenotypes will experience reduced vulnerability to lethal top predators and reduced foraging efficiency relative to individuals without extreme predator-induced phenotypes. Fourth, I hypothesize that intermediate predators with extreme competitor-induced phenotypes will be more proficient in obtaining prey but more vulnerable to predation by top predators. Finally, intermediate predators from extreme competitive larval environments with predators will perform less effectively than extreme phenotypes (extreme competitive and extreme predation larval environments) due to conflicting developmental constraints from predator and competitor cues simultaneously.

Methods

Study system

Overwintered dragonfly naiads, *Anax* spp, were the top predators in this study and intermediate predators were larvae of the salamander, *Ambystoma opacum*. *Anax* naiads are voracious predators of larval amphibians in temporary pond communities (Van Buskirk 1988, Wilbur and Fauth 1990, Relyea 2007). *A. opacum* are common predators in temporary pond communities of the eastern U.S. and can function as a keystone predator of larval anurans in pond communities (Morin 1995, Petranka 1998, Chalcraft & Resetarits 2003). Previous work suggests that *A. opacum* will elicit responses to cues from *Anax* since other larval ambystomatid salamanders have been found to alter morphological and behavioral traits in response to dragonfly predators (Storfer and White 2004, Yurewicz 2004). Larval *A. opacum* can never reach a size refuge to avoid consumption by *Anax* and therefore are susceptible to *Anax* throughout their entire larval period (Relyea & Yurewicz 2002, Davenport, personal observation). Both *Anax* and *A. opacum* consume a shared prey resource consisting of macroinvertebrates and small larval anurans (e.g., *Bufo*, *Pseudacris*,*Rana*) thus completing the intraguild food web in pond communities (Chalcraft & Resetarits 2003, Morin 1995, Van Buskirk 1988, Wilbur & Fauth 1990).

I conducted three experiments during the course of this study. In the first experiment, I measured the behavioral and morphological responses of intermediate predators (larval salamanders; *Ambystoma opacum*) to the presence of a nonlethal top predator (larval dragonflies; *Anax* spp.) in environments that differ in the abundance of intermediate predators present. I measured responses during the early, middle and late stages of larval salamander development. This experiment will hereafter be referred to as the "plasticity experiment". For the second and

third experiment, I assessed how four of the different intermediate predator phenotypes produced in the plasticity experiment differed in their ability to 1) escape predation and 2) forage for food resources. I will hereafter refer to this set of experiments as "performance trials". The second experiment assessed the foraging efficiency of different *A. opacum* phenotypes and the third experiment evaluated how vulnerable different *A. opacum* phenotypes were to predation by larval *Anax*.

Plasticity Experiment

This experiment was conducted in mesocosms made from 1100 l (1.9 m surface area) cattle tanks designed to mimic natural ponds (Morin 1981). Mesocosms represent a quasi-natural setting for ecologists to conduct experiments without compromising complexity and replication (Morin 1989, Wilbur 1989). More importantly, ecologists have found that the same processes found to be important to aquatic organisms in mesocosms are also important in natural settings (Resetarits and Fauth 1998, Rubbo et al. 2006, Van Buskirk and McCollum 1999, Van Buskirk 2009). Six treatments were established in mesocosms for this experiment resulting from all possible combinations of two levels of a predator manipulation involving larval *Anax* (2 empty cages or 2 cages with one nonlethal *Anax* each) crossed with three levels of larval *A. opacum* density (10 , 20, or 40 individuals). All *A. opacum* densities used in this experiment ($5/m^2$-$20/m^2$) are comparable to natural densities of *A. opacum* ($12\text{-}47/m^2$; Smith 1988, Petranka 1989) observed in the field or from literature records.

Each of the six treatments was randomly assigned to one mesocosm within each of four spatial blocks for a total of 24 mesocosms. Mesocosms were located at the West Research Campus of East Carolina University. Each mesocosm was equipped with a standpipe to control

water levels and fiberglass mesh lids to prevent study animals from escape and unwanted non-study animal colonization. Mesocosms were filled with well water on 16 November 2007 and filled with one kg of leaf litter on 18 November 2007. Aliquots of concentrated zooplankton from local ponds were added to each mesocosm on 27 November 2007. I collected *A. opacum* eggs from the Croatan National Forest on 4-7 November 2007 and hatching was induced on 29 November 2007. Larval *Anax* were collected from the Croatan National Forest on 29 November 2007. All study animals were randomly assigned to their respective treatments and placed into mesocosms on 6 December 2007 to begin the experiment. All tanks started with 50 cm of water and then experienced a drying regime of 178 days which is representative of *A. opacum* larval period. The drying regime was based on field observations (from eastern NC) and prior literature and developed from the methods of Wilbur (1987).

Cages have been successfully utilized by other researchers in aquatic studies to understand non-consumptive effects of predators on their prey (McCollum and Van Buskirk 1996, Blaustein 1997, Benard 2004, Relyea 2007). To quantify the non-consumptive effects of *Anax* on *A. opacum*, I equipped all tanks with two PVC cages (10 cm x 10 cm). Tanks assigned to a caged predator treatment received two cages that each contained one *Anax*, while tanks assigned to treatments designated to have no predators received two empty cages. Caged *Anax* were fed a single larval salamander every three days until the completion of the experiment. All tanks without caged *Anax* were also lifted from the bottom of the tank (as this was necessary to feed caged *Anax*) when caged *Anax* were fed to account for any differences in disturbance due to feeding of the caged *Anax*.

Morphological measurements were taken three times during the larval period of *A. opacum*. The purpose of dividing up the larval period of *A. opacum* into thirds was to document

treatment differences in the morphological traits of *A. opacum* at different stages of ontological development. The measurements were taken on the following three sampling periods: 30 January-2 February 2008, 26-28 March 2008, and 12-13 May 2008. During each sampling period, I captured 40% of the individuals in each mesocosm with a dip net and photographed the lateral and ventral side of each salamander so that I could measure salamander traits from digital images. To facilitate photography, I placed captured individuals in an Orajel® solution for anesthetization (Cecala et al. 2007) and then placed individual animals into a photo chamber (as described in Van Buskirk and Schmidt 2000). The photo chamber was equipped with a scale and mirrors that allowed me to take a photograph of the side and venter of a larval salamander simultaneously. Each captured larval salamander was photographed, weighed, and then placed back into a container of fresh pond water. After approximately 4-7 minutes, all larval salamanders had recovered and were returned to the experimental tank from which they were taken. No mortality was experienced during the photographing sessions. ImageJ was used to measure: 1) head length; 2) head depth; 3) head width; 4) torso length; 5) tail length; 6) tail fin depth; 7) tail muscle depth; 8) tail muscle width for each photograph of a salamander (Van Buskirk and Schmidt 2000).

Behavioral observations were made only during the first sampling period (30 January-2 February) because mesocosms became too murky (after 21 March) to make accurate observations of salamanders in the majority of mesocosms. Behavior was assessed by recording the number of larval *A. opacum* that were active and the total number of larval *A. opacum* observed with scan sampling technique (Altmann 1974) in a given tank. Each tank was observed for 10-15 seconds every 6 hours for a 24 hour period. I paired each set of the four observations (the number of active *A. opacum* divided by the number of observed *A. opacum*

during this 24 hour period in each tank) to calculate an average activity ratio for a given tank. By dividing the mean number of active *A. opacum* in each tank by the mean number of observed *A. opacum,* I was able to get a measure of the proportion of individuals that were active in tanks.

Performance Trials

In order to examine the performance of *A. opacum* associated with predator- and competitor-induced phenotypes, I set up 32 mesocosms on 17 November 2009 to induce phenotypes observed in four of the six larval environments considered in the plasticity experiment. I focused on four environments rather than all six environments considered for the plasticity experiment due constraints on the number of mesocosms available. I chose the most extreme larval environments from the plasticity experiment: 1) 10 *A. opacum*, no caged *Anax*, 2) 10 *A. opacum*, caged *Anax*, 3) 40 *A. opacum*, no caged *Anax*, and 4) 40 *A. opacum*, caged *Anax*. The extent of replication of each larval environment was based on how many individuals (a minimum of 64 individuals of each phenotype) from each larval environment would be needed to complete the performance trials on salamander vulnerability and foraging efficiency. Of the 32 mesocosms established, 24 mesocosms (12 with caged *Anax* and 12 without caged *Anax*) had 10 newly hatched *A. opacum* and 8 mesocosms (4 with caged *Anax* and 4 without caged *Anax*) had 40 newly hatched *A. opacum*. My prior work suggests that this should produce more than enough individuals (assuming a low survival of 50% for each phenotype) to perform tests on foraging efficiency and predator vulnerability.

The methodology for creating these mesocosms was identical to that in the plasticity experiment. Leaf litter (1 kg of hardwood) and pond water aliquots were randomly assigned to mesocosms on 20-22 November 2009. *A. opacum* nests were collected on 3-10 November 2009

and hatching was induced beginning on 19 November 2009. Larval *Anax* were collected on 23 November 2009. The experiment began on 1 December 2009 after all tanks had been randomly assigned treatments and study organisms. Caged larval dragonflies were fed every three days until week 22 of larval *A. opacum* development. Based on the plasticity experiment, week 17 is when *A. opacum* express maximum differences in morphological traits; however I chose to wait and conduct performance trials until week 22, because growth rates of *A. opacum* were retarded when compared to the plasticity experiment. *A. opacum* growth rates were likely hindered by an unusually cold winter in eastern North Carolina; therefore I monitored *A. opacum* growth by randomly selecting individuals and measuring masses until masses were comparable to that observed to week 17 in the plasticity experiment before performance trials began.

Methods for assessing foraging efficiency

I measured the foraging efficiency of 10 individuals from each of the four larval environments. Thus this experimental design is comprised of 4 treatments (phenotypes from the 4 larval environments) that were replicated 10 times. To measure the foraging efficiency of *A. opacum*, I placed one individual within a 31 liter (L) (52.1 cm x 36.1 cm x 30.7 cm) tub filled with filtered pond water. I held the density of *A. opacum* in the foraging trials constant so that I can ensure that any differences among treatments are due to differences in morphology rather than density. Independent manipulations of density along with phenotype for foraging trials would be very difficult to perform for logistical reasons (e.g., need to maintain twice as many animals and experimental tubs within a very short time interval). Each tub had 20 grams of leaf litter at the bottom to provide a more natural substrate. Prey items for *A. opacum* in each tub were 40 *Daphnia* spp. Tubs were located outdoors at the West Research Campus of East

Carolina University. After 24 hours of feeding, I removed the *A. opacum* and washed all leaf litter to remove all *Daphnia* adhering to leaf litter. I also filtered the water in each tub (including the wash water) through a series of sieves (500 and 250 μm) to retrieve any remaining prey items. Ten sets of tubs that did not have *A. opacum* in them were established to measure the efficiency at which I can extract *Daphnia* from the tank. Foraging efficiency for *A. opacum* was defined as the difference between the number of *Daphnia* that are successfully removed from tubs without *A. opacum* and the number of *Daphnia* successfully removed from tubs with *A. opacum*.

Methods for assessing vulnerability to top predators

I conducted a second experiment to measure the vulnerability of the four *A. opacum* phenotypes to *Anax*. I measured the vulnerability of each phenotype by measuring the average number of each phenotype that is successfully captured and killed by a single *Anax* in a 31 L tub over a 24 hour period. Each experimental tub was equipped with 30 g of leaf litter. I measured the efficiency at which *Anax* captures each *A. opacum* phenotype twelve times (only 11 times for the 10 *A. opacum* with caged *Anax* phenotype due to a limited supply of individuals for trials). To measure capture efficiency, I placed 5 *A. opacum* of a particular phenotype into a tub along with a single *Anax*. A density of 5 individuals in this experiment instead of 1 individual is to insure that some individuals survive for measurement. As before, I held the density of *A. opacum* constant so that I can ensure that any differences among treatments are due to differences in morphology rather than density. As with the foraging trials, independent manipulations of density along with phenotype would be very difficult to perform for logistical

reasons (e.g., maintenance of animals and experimental tubs within a very short time interval). I removed and counted all surviving animals at the end of the trial period.

Statistical Analyses

I performed a factorial ANOVA on ln-transformed survival and mean mass of *A. opacum* with the two following main factors and their interaction; 1) *A. opacum* density and 2) presence of caged *Anax* cues in the larval environment. A separate factorial ANOVA for *A. opacum* mean mass was conducted for each of the three sampling periods (early, mid, late). All morphological and mass measurements were log-transformed to normalize residuals for statistical analyses. After transformation, I analyzed *A. opacum* morphology with ANCOVA which included treatment as a categorical variable and mass as a covariate. Post hoc comparisons of mean trait values were also made in ANCOVA (using Ryan-Einot-Gabriel-Welsch multiple range test) to detect treatment differences among groups. The ANCOVA model included a term for the interaction between treatment and mass in order to test the hypothesis that the allometric relationship between mass and morphology was the same across the six treatments. Proportional activity data were arcsine-transformed and analyzed with a factorial ANOVA with the main effects of *A. opacum* density and predators as well as the interaction between *A. opacum* density and predator presence. Block effects were included in all analyses for the plasticity experiment.

I performed a factorial ANOVA on data from the performance trials to evaluate whether the larval environment that *A. opacum* was raised in until week 22 affects *A. opacum* foraging efficiency or vulnerability of *A. opacum* to free-swimming, lethal *Anax*. The factorial model included the independent and interactive effects of predators (caged *Anax* presence or absence)

and conspecifics (10 versus 40 *A. opacum* present) in the larval environment in which larval *A. opacum* were raised.

The response variable for foraging efficiency performance trials was the difference in number of *Daphnia* recovered without *A. opacum* minus the number of *Daphnia* recovered with *A. opacum.* For the vulnerability performance trial, the ln-transformed proportion of surviving *A. opacum* of each different phenotype was the response variable. Block effects accounted for little variation in responses in the performance trials ($P > 0.190$) which suggests that blocking was not an efficient scheme for assessing treatment effects in the performance trials. Thus, removal of block effects from ANOVA models associated with the performance trials would enhance the statistical power for evaluating treatment effects in both of the performance trials. Given that the interpretation of ANOVA results are different whether block effects are included in the model (treatment effects are less likely to be documented in these scenarios when block effects are included), I present the results from each factorial ANOVA with and without block effects for the performance trials so that readers can reach their own conclusions.

Results

Plasticity Experiment

Survival of *A. opacum* during the experiment was reduced by conspecific density ($F_{2,6} = 6.55$, $P = 0.031$; Fig 6) and the presence of caged *Anax* ($F_{1,6} = 8.90$, $P = 0.058$; Fig 6). The interactive effects between conspecific density and caged *Anax* ($F_{2,6} = 0.10$, $P = 0.910$; Fig 6) did not have an impact on *A. opacum* survival. Increasing conspecific density reduced *A. opacum* mass during each sampling period (sampling period 1; $F_{2,6} = 14.43$, $P = 0.005$, sampling period 2; $F_{2,6} = 34.93$, $P < 0.001$, sampling period 3; $F_{2,6} = 5.25$, $P = 0.048$). *A. opacum* mass was not

affected by the presence of caged *Anax* during any sampling period (sampling period 1; $F_{1,6}$ = 1.94, P = 0.258, sampling period 2; $F_{1,6}$ = 0.01, P = 0.918, sampling period 3; $F_{1,6}$ = 1.25, P = 0.346) or by the interaction between conspecific density and caged *Anax* presence during any sampling period (sampling period 1; $F_{2,6}$ = 0.98, P = 0.427, sampling period 2; $F_{2,6}$ = 0.38, P = 0.697, sampling period 3; $F_{2,6}$ = 0.43, P = 0.670).

As expected, the morphological traits of salamanders were always bigger in larger salamanders regardless of sampling period (log mass variable in Table 2a-c). This is indicated by the significant covariate (mass) effects in all ANCOVA models (Table 2a-c). Treatments did not produce variation in any morphological trait during the early (Table 2a; Figs B-1-B-8 in Appendix B) or late (Table 2c; Figs B-15-B-22 in Appendix B) stages of larval development beyond that which was attributable to differences in body mass among treatments, nor did they alter the allometric relationship between any morphological trait and mass. Induced morphological responses were expressed during the middle of the larval period for some morphological traits (tail fin depth and torso length; Table 2b) but not all eight morphological traits measured (Table 2b; Figs B-9-B14 in Appendix B). *A. opacum* tail fin depth was significantly altered independently of treatment imposed differences in body mass (Table 2b). Post hoc comparisons demonstrate that the average *A. opacum* tail fin depths at the middle sampling period were statistically different from each other across four of the six treatments, with the exception of *A. opacum* tail fin depths in low and intermediate conspecific densities exposed to caged predator cues not being statistically different from one another during the middle sampling period (Fig. 7). Treatment did not have a detectable effect on the slope of the allometric relationship between *A. opacum* tail fin depth and *A. opacum* mass (Table 2b).

The other trait that was significantly affected by the larval environment was *A. opacum* torso length (Table 2b). Treatment significantly affected the slope of the allometric relationship between *A. opacum* torso length and *A. opacum* mass (Table 2b). Given that *A. opacum* mass differed among conspecific density treatments but not with predator treatments, I compared expected values of morphological traits between predator treatments within each density treatment for the average sized individual within each density treatment. Expected values of morphological traits (and their estimate of variability) for the average sized individual in a particular density treatment was derived for each treatment from the allometric relationship for each treatment. Expected values for the average sized individual within each density treatment were compared between the two levels of the predator treatment with a t-test. There were no detectable differences in torso length of the average sized *A. opacum* in caged predator treatments and in predator free treatments when there was an intermediate (t_6=0.62, P = 0.551) or high (t_6=-0.17, P = 0.868) abundance of conspecifics present. There was a significant difference, however, in torso length of the average sized *A. opacum* in caged predator treatments vs. average sized *A. opacum* in no caged predator treatments in low conspecific density environments (t_6=3.31, P = 0.009). Specifically, *A. opacum* exposed to caged predator cues had shorter torsos than those not exposed to *Anax* cues in low conspecific density environments (Fig. 8).

During early stages of development, the effect of *Anax* on *A. opacum* activity was dependent on *A. opacum* density in the environment ($F_{2,6}$ = 5.79, P = 0.039; Fig.9). Specifically, activity levels were consistently low with caged *Anax* regardless of conspecific density; however as *A. opacum* densities increased without caged *Anax*, less *A. opacum* were active. Thus at low densities, *A. opacum* are much more active when caged *Anax* are not present and this appears to

be driving this interactive effect of caged *Anax* and *A. opacum* density. *A. opacum* reduced activity levels in the presence of caged *Anax* ($F_{1,6}$ = 12. 07, P = 0.040; Fig.9) but activity levels were not influenced by *A. opacum* density ($F_{2,6}$ = 2.59, P = 0.154; Fig.9).

Performance Trials

Predator-induced phenotypes of *A. opacum* tended to be less vulnerable to lethal *Anax* than non-predator-induced phenotypes (with block effects: $F_{1,10}$ = 3.31, P = 0.099, without block effects: $F_{1,43}$ = 6.52, P = 0.014; Fig. 10). Competitor-induced phenotypes from high conspecific environments were more vulnerable to top predators, *Anax*, than phenotypes that arise in the environments with few competitors (with block effects: $F_{1,10}$ = 6.83, P = 0.024, without block effects: $F_{1,43}$ = 6.52, P = 0.014; Fig. 10). Despite having opposing effects on *A. opacum* vulnerability, changes in the phenotypes of *A. opacum* that were due to the presence of predators and competitors resulted in additive changes in vulnerability to predation (with block effects: $F_{1,10}$ = 0.02, P = 0.897, without block effects: $F_{1,43}$ = 0.14, P = 0.714; Fig. 10).

Predator-induced phenotypes did not differ from non-predator-induced phenotypes in their foraging efficiency (with block effects: $F_{1,9}$ = 3.10, P = 0.112, without block effects: $F_{1, 36}$ = 2.53, P = 0.120; Fig. 11). Furthermore, phenotypes derived in environments with a high density of conspecifics were just as efficient in their foraging as phenotypes derived from environments with a low density of conspecifics (with block effects: $F_{1,9}$ = 0.27, P = 0.618, without block effects: $F_{1,36}$ = 0.25, P = 0.622; Fig. 11). The simultaneous exposure of *A. opacum* to both predators and a high abundance of conspecifics during the larval environment did not result in a change in the foraging efficiency of *A. opacum* that would otherwise be expected by the

independent influence of predators and higher densities of competitors (with block effects: $F_{1,9}$ = 0.37, P = 0.556, without block effects: $F_{1,36}$ = 0.30, P = 0.588; Fig. 11).

Discussion

A. opacum responded to the presence of *Anax* cues by altering some aspects of their morphology and activity levels, but the magnitude of response was dependent on the ontological stage of development of the animal and the density of conspecifics present. Activity levels of *A. opacum* were always reduced in the presence of caged *Anax* during early stages of larval development, but I was unable to assess behavior during later stages of larval development. Differences in *A. opacum* morphological traits were only observed during the middle stages of larval development. *A. opacum* do not express the shorter bodies and deeper tails until the middle of their larval period (Fig. 12). Shorter bodies were dependent on predator cues being present and conspecific densities that are low enough for the individuals to respond (Fig. 13). Values for another trait, tail length, was not statistically distinguishable among treatments during the middle of the larval period, but tended to be shorter in individuals that were exposed to predator cues at all *A. opacum* densities. All of these morphological responses appear to confer an advantage for predator-induced *A. opacum* by reducing their short-term vulnerability to free swimming, lethal *Anax*. Surprisingly, I did not find a foraging efficiency trade-off associated with morphological responses during my performance trials, however there was a trend for fewer *Daphnia* to be recovered from tubs with non-predator induced phenotypes of *A. opacum* than in tubs with predator-induced phenotypes of *A. opacum*.

The two traits (deeper tail fins and shorter bodies) that I found to be significantly affected by cues in the environment have been found to be important for larval amphibian locomotion

(McCollum and Leimberger 1997, Van Buskirk and McCollum 2000). Deeper tail fins provide tadpoles, small fish and larval salamanders with improved propulsion and maneuverability (Webb 1984, Dommenici and Blake 1997), and can translate into faster starts (from the "C-start") and swim speeds for larval anurans (Wassersug and Hoff 1985, Wassersug 1989, Landberg and Azizi 2010). Shorter bodies are likely induced to provide predators, especially striking predators, with smaller targets since strikes can be deadly to that area (Van Buskirk et al. 2003). Tail length did tend to be shorter when individuals were exposed to predator cues and may have also been to provide predators with a smaller target.

Three other studies have considered larval salamander morphology in response to predators and found that additional morphological traits were induced (Van Buskirk and Schmidt 2000, Storfer and White 2004, Yurewicz 2004). Specifically, they found that salamanders developed larger heads and larger tail muscles in response to predator cues. I did not find a component of either of these two morphological traits (head or tail muscle measurements) to be statistically distinguishable during this experiment with *A. opacum*. I was surprised by the lack of variation in head traits since other Ambystomatid and Asian salamanders have been found to have larger heads leading to cannibalism (Collins and Cheek 1983, Nishihara 1996, Maret and Collins 1997, Michimae and Wakahara 2001). Larger heads would allow those individuals to consume larger prey, more prey items and even intraspecific competitors especially at high densities (Loeb et al. 1994, Yurewicz 2004). Head traits did not differ among treatments and survival was also relatively high across all treatments, therefore it appears as if this did not occur during this experiment. Although other studies were with congeneric salamanders (Maret and Collins 1997, Storfer and White 2004, Yurewicz 2004), I hypothesize that *A. opacum* responded differently to the environment and did not display these morphological changes since it has a

significantly longer larval period than all other pond-breeding salamanders previously studied (8 months compared to 3-5 months). The larval period of *A. opacum* is longer since it overwinters in ponds and experiences slower growth rates during a significant portion of its' larval period in comparison to other ambystomatid salamanders (Petranka 1998). Some *A. opacum* morphological traits (e.g. head, tail muscle) may not be expressed due to balancing the conflicting constraints placed on individual salamanders during the late fall/winter growing season.

An increase in survival for predator-induced phenotypes has been found in previous studies with other taxa (Tollrian and Harvell 1999, Miner et al. 2005). Generally, individuals exposed to nonlethal predator cues express a phenotype that has lower vulnerability to a lethal predator when compared to individuals that were not exposed to nonlethal predator cues. I found that vulnerability was lowest for larval salamanders at low *A. opacum* densities and caged *Anax* cues. However, vulnerability of salamanders in environments with high conspecific densities and caged *Anax* cues was not statistically distinguishable from the vulnerability of salamanders from low competitor densities and no caged *Anax* cues environments. This supports the hypothesis that individuals exposed to predator cues and competitors would experience some constraint in expressing an effective predator-induced phenotype and therefore would suffer in lethal predator environments. This suggests that the additive effects of *A. opacum* density and caged *Anax* cues are preventing *A. opacum* from expression of an effective predator-induced phenotype at high conspecific densities possibly due to resource competition. Vulnerability was highest for the *A. opacum* phenotypes from high conspecific density and no caged *Anax* cue environments in the vulnerability trials with free-swimming, lethal *Anax*.

Resources may have been lower in high conspecific environments due to increased resource competition. In such environments, *A. opacum* may be forced to forgo predator defenses just in order to persist. I did not monitor zooplankton abundances during this experiment, but I did note that tanks with high *A. opacum* densities did have earlier algal blooms than tanks with low *A. opacum* densities (Davenport, unpublished data). In foraging trials, *A. opacum* without *Anax* cues did not consume more zooplankton than *A. opacum* with *Anax* cues (Fig. 10). However, there was a trend for predator-induced phenotypes to consume fewer *Daphnia* than non-predator-induced phenotypes during the 24 hour period (Fig. 10). The foraging performance trials may not support the original hypothesis that a predator phenotype carries a cost. Nonetheless I feel that a cost is still likely. An increase in replication may have led to a more robust statistical comparison of foraging efficiency among treatments.

A novel aspect of this study is that morphological responses to treatments were documented throughout the *A. opacum* larval period (but see Van Buskirk and Schmidt 2000). The fact that morphological responses to treatments were only detectable during the middle stages of larval development (day 113) supports the hypothesis that morphological traits take time to develop (Hoverman and Relyea 2007). Previous research has found that tadpoles and freshwater snails also require a minimum window of time before morphological defenses can be induced (Van Buskirk 2002, Hoverman and Relyea 2009). Most plasticity studies with amphibians, however, have focused on induction of traits within the first month of exposure to predator cues, hence *A. opacum* morphological defenses would have been missed since induction of traits did not occur until the middle of the larval period (day 113). This delay in response is likely due to a lag in the allocation of tissues away from growth and towards shorter bodies and deeper tails. During the lag in time to induction of morphological defenses, many species may

rely on behavioral responses to avoid predation (Relyea 2003). I found that during early stages of development when morphological differences among *A. opacum* were rather minor, *A. opacum* did alter their behavior in response to the environment. Thus, *A. opacum* does seem to rely on behavioral responses to some degree during the early larval period and may throughout the rest of the larval period. Unfortunately, I was unable to measure behavior after the early sampling period due to murky pond water in mesocosms.

Interestingly, *A. opacum* phenotypes were not statistically distinguishable among treatments just before metamorphosis. The disappearance of differences in *A. opacum* morphology among treatments just prior to metamorphosis could be due to two interrelated reasons. First, salamander morphological responses were found in the trunk and tail which may all converge before metamorphosis due to developmental constraints. Unlike tadpoles, salamanders retain their tails after metamorphosis but their tail fins are absorbed and reduced. This suggests that no matter how tall tail fins are during the larval period of a salamander there is a restriction once they metamorphose. Second, it is possible that there is a minimum torso length that must be reached before salamanders can initiate metamorphosis. Hence, salamanders with shorter torsos likely enhanced torso growth during the latter part of the larval period by reallocating the energy (tissues) from their expressed tall tail fins to torsos. Convergence may also coincide with reduced mortality risk from *Anax* consumption since *A. opacum* approaches a size that is not easily consumed by *Anax* during the latter stage of the larval period. *Anax* may not selectively consume larger *A. opacum*, but *Anax* are capable of consuming all size classes (Davenport, unpublished data). *Anax* may have more difficulty with larger and less common prey types (Bergelson 1985) likely due to higher energy expenditure when trying to capture

larger *A. opacum*. These two interrelated reasons would help explain, along with a minimum prerequisite for metamorphosis, the convergence of *A. opacum* phenotypes.

Intermediate predators with predator-induced defenses can persist for longer periods of time with top predators than intermediate predators not exposed to top predators (Kratina et al. 2010). However, work here suggests that ecologists should consider that intermediate predators are often entangled in complex food webs where they are exposed to competition and predation. Traits influenced by predation are also influenced by the density of intraspecific competitors in the system and this may alter the outcomes of prior studies that only considered predator-induced traits and their benefits. Intermediate predators experience conflicting signals from the environment that prevents them from fully inducing the phenotype that matches the environment. Additionally, this work highlights the importance of ontogenetic changes in traits, especially given that certain induced traits may be influenced by intraspecific competitors and therefore a decrease in the likelihood of trait induction. This is vital since intermediate predators with complex life histories, such as found in pond systems, can decrease vulnerability during their larval period (in ponds) when compared to other larval organisms by inducing defenses and then metamorphose out of those larval habitats to the next life stage.

References

Abrams, P.A. 1990. The evolution of antipredator traits in prey in response to evolutionary change in predators. Oikos 59:147-156.

Abrams, P.A. 2008. Measuring the impact of dynamic antipredator traits on predator-prey-resource interactions. Ecology 89:1640-1649.

Abrams, P.A., C. Hill, and R. Elmgren. 1990. The functional-response of the predatory polychaete *Harmothoe sarsi*, to the amphipod, *Pontopreia affinis.* Oikos 59:261-269.

Arim, M. and P. Marquet. 2004. Intraguild predation: a widespread interaction related to species biology. Ecology Letters 7:557-564.

Ashton, I.W., A.E. Miller, W.D. Bowman, and K.N. Suding. 2010. Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. Ecology 91:3252-3260.

Benard, M.F. 2004. Predator-induced phenotypic plasticity in organisms with complex life histories. Annual Review of Ecology, Evolution and Systematics 35:651-673.

Berg, M.P. and J. Ellers. 2010. Trait plasticity in species interactions: a driving force of community dynamics. Evolutionary Ecology 24:617-629.

Blaustein, L. 1997. Non-consumptive effects of larval *Salamandra* on crustacean prey: can eggs detect predators? Oecologia 110:212-217.

Boeing, W.J. and C.W. Ramcharan. 2010. Inducible defences are a stabilizing factor for predator and prey populations: a field experiment. Freshwater Biology 55:2332-2338.

Cecala, K.K., S.J. Price, and M.E. Dorcas. 2007. A comparison of MS-222 (Tricaine Methane Sulfonate) and Orajel® as amphibian anesthesia. Herpetological Review 38:63-66.

Collins, J.P. and J.E. Cheek. 1983. Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. American Zoologist 23:77-84.

Dommenici, P. and R.W. Blake. 1997. The kinematics and performance of fish fast-start swimming. Journal of Experimental Biology 200:1165-1178.

Gabriel, W. 1999. Evolution of reversible plastic responses: inducible defenses and environmental tolerance. Pages 286-305 *in* R. Tollrian and D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, USA.

Gabriel, W., B. Luttbeg, A. Sih, and R. Tollrian. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. American Naturalist 166:339-353.

Holt, R.D. and G.A. Polis. 1997. A theoretical framework for intraguild predation. American Naturalist 149:745-764.

Hoverman, J.T. and R.A. Relyea. 2007. How flexible is phenotypic plasticity? Developmental windows for the induction and reversal of inducible defenses. Ecology 88:693-705.

Hoverman, J.T. and R.A. Relyea. 2009. Survival trade-offs associated with inducible defenses in snails: the roles of multiple predators and developmental plasticity. Functional Ecology 23:1179-1188.

Kratina, P., E. Hammill, and B.R. Anholt. 2010. Stronger inducible defenses enhance persistence of intraguild prey. Journal of Animal Ecology 79:993-999.

Landberg, T. and E. Azizi. 2010. Ontogeny of escape swimming performance in the spotted salamander. Functional Ecology 24:576-587.

Lima, S.L. 1998. Nonlethal effects in the ecology of predator-prey interactions. Bioscience 48:25-34.

Lima, S.L. and L.M. Dill. 1990. Behavioral decisions made under the risk of predation; a review and prospectus. Canadian Journal of Zoology 68:619-640.

Loeb, M.L.G., J.P. Collins, and T.J. Maret. 1994. The role of prey in controlling expression of a trophic polymorphism in *Ambystoma tigrinum nebulosum*. Functional Ecology 8:151-158.

Maret, T.J. and J.P. Collins. 1997. Ecological origins of morphological diversity: a study of alternative trophic phenotypes in larval salamanders. Evolution 51:898-905.

McCoy, M.W. 2007. Conspecific density determines the magnitude and character of predator-induced phenotype. Oecologia 153:871-878.

McCollum, S.A. and J. Van Buskirk. 1996. Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. Evolution 50:583-593.

McCollum, S.A. and J.D. Leimberger. 1997. Predator-induced morphological changes in an amphibian: Predation by dragonflies affects tadpole shape and color. Oecologia 109:615-621.

Michimae, H. and M. Wakahara. 2001. Factors which affect the occurrence of cannibalism and the broad headed "cannibal" morph in larvae of the salamander *Hynobius retardatus*. Behavioral Ecology and Sociobiology 50:399-345.

Miner, B.G., S.E. Sultan, S.G. Morgan, D.K. Padilla, and R.A. Relyea. 2005. The ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution 20:685-692.

Morin, P.J. 1981. Predatory salamanders reverse the outcome of competition among three species of anuran tadpoles. Science 212:1691.

Morin, P.J. 1989. New directions in amphibian community ecology. Herpetologica 45:124-128.

Mougi, A. and O. Kishida. 2009. Reciprocal phenotypic plasticity can lead to stable predator-prey interaction. Journal of Animal Ecology 78:1172-1181.

Nishihara, A. 1996. Effects of density on growth of head size in larvae of the salamander *Hynobius retardatus*. Copeia 1996:478-483.

Pangle, K.L. S.D. Peacor, and O.E. Johannsson. 2007. Large nonlethal effects of an invasive invertebrate predator on zoolplankton population growth rate. Ecology 88:402-412.

Peacor, S.D. 2003. Phenotypic modifications to conspecific density arising from predation risk assessment. Oikos 100:409-415.

Petranka, J.W. 1989. Density-dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. Ecology 70:1752-1767.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C.

Polis, G.A. and R.D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends in Ecology and Evolution 7:151-154.

Polis, G.A., C.A. Myers, and R.D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297-330.

Preisser, E.I., D.I. Bolnick, and M.F. Benard. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86:501-509.

Relyea, R.A. 2002. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. Ecological Monographs 72:523-540.

Relyea, R.A. 2003. Predators come and go: the reversibility of predator-induced traits. Ecology 84:1840-1848.

Relyea, R.A. 2007. Getting out alive: how predators affect the decision to metamorphose. Oecologia 152:389-400.

Relyea, R.A. and J.R. Auld. 2004. Having the guts to compete: How intestinal plasticity explains the cost of inducible defenses. Ecology Letters 7:869-875.

Resetarits, W.J., Jr. and J.E. Fauth. 1998. From cattle tanks to Carolina bays: the utility of model systems for understanding natural communities. Pages 133-151 in W.J. Resetarists, Jr. and J. Bernardo, editors. Experimental Ecology: issues and perspectives. Oxford University Press, New York, New York, USA.

Rubbo, M. J., K. Shea, and J.M. Kiesecker. 2006. The influence of multi-stage predation on population growth and the distribution of the pond-breeding salamander, *Ambystoma jeffersonianum*. Canadian Journal of Zoology 84:449-458.

Schlichting, C.D. and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, Massachusetts, USA.

Schoeppner, N.M. and R.A. Relyea. 2009. When should prey respond to heterospecific alarm cues? Testing the hypotheses of perceived risk. Copeia 2009:190-194.

Scott, D.E., E.D. Casey, M.F. Donovan, and T.K. Lynch. 2007. Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. Oecologia 153:521-532.

Smith, C.K. 1988. Ecological significance of size variation in the marbled salamander, *A. opacum*. Dissertation, University of North Carolina, Chapel Hill, North Carolina, USA.

Storfer, A. and C. White. 2004. Phenotypically plastic responses of larval tiger salamanders, *Ambystoma tigrinum*, to different predators. Journal of Herpetology 38:612-615.

Teplitsky, C. and A. Laurila. 2007. Flexible defense strategies: competition modifies investment in behavioral vs. morphological defenses. Ecology 88:1641-1646.

Tollrian, R. and D. Harvell. 1999. The ecology and evolution of inducible defenses. Princeton University, Princeton, New Jersey, USA.

Van Buskirk, J. 2002. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. American Naturalist 160:87-102.

Van Buskirk, J. 2009. Natural variation in morphology of larval amphibians: Phenotypic plasticity in nature? Ecological Monographs 79:681-705.

Van Buskirk, J. and R.A. Relyea. 1998. Natural selection for phenotypic plasticity: predator-induced morphological responses in tadpoles. Biological Journal of the Linnean Society 65:301-328.

Van Buskirk, J. and S.A. McCollum. 1999. Plasticity and selection explain variation in tadpole phenotype between ponds with different predator composition. Oikos 85:31-39.

Van Buskirk, J. and S.A. McCollum. 2000a. Influence of tail shape on tadpole swimming performance. Journal of Experimental Biology 203:2149-2158.

Van Buskirk, J. and S.A. McCollum. 2000b. Functional mechanisms of an inducible defense in tadpoles: morphology and behavior influence mortality and risk from predation. Journal f Evolutionary Biology 13:336-347.

Van Buskirk, J. and B.R. Schmidt. 2000. Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. Ecology 81:3009-3028.

Van Buskirk, J., P. Anderwald, S. Lupold, L. Reinhardt, and H. Schuler. 2003. The lure effect, tadpole tail shape, and the target of dragonfly strikes. Journal of Herpetology 37:420-424.

Van Buskirk, J., M. Ferrari, D. Kueng, K. Näpflin, and N. Ritter. *In press*. Prey risk assessment depends on conspecific density. Oikos.

Wassersug, R.J. 1989. Locomotion in amphibian larvae (or, "why aren't tadpoles built like fishes?"). American Zoologist 29:65-84.

Wassersug, R.J. and K.S. Hoff. 1985. The kinematics of swimming in anuran larvae. Journal of Experimental Biology 119:4-30.

Webb, P.W. 1984. Body form, locomotion, and foraging in aquatic vertebrates. American Zoologist 24:107-120.

Wilbur, H.M. 1987. Regulation of structure in complex systems: Experimental temporary pond communities. Ecology 68:1437-1452.

Wilbur, H.M. 1989. In defense of tanks. Herpetologica 45:122-123.

Yurewicz, K.L. 2004. A growth/mortality trade-off in larval salamanders and the coexistence of intraguild predators and prey. Oecologia 138:102-111.

Table 2. Analysis of covariance results for morphological responses of *Ambystoma opacum* to six different larval environments varying in the occurrence of caged *Anax* predators and densities of conspecifics present) during the A) early stages (day 59), B) middle stages (day 113) and C) late stages of larval development. Data in the table are the F values followed by the *p* value in parentheses. Bold numbers and traits indicate treatment statistical significance. Degrees of freedom for: Block = 3,9, Treatment = 5, 9, Log mass = 1,9, and Treatment X Log mass = 5, 9 in all cases.

	Source of variation			
A) Early Response	Block	Treatment	Log mass	Treatment X Log mass
Log head length	5.71 (0.018)	1.30 (0.344)	21.52 (0.001)	1.02 (0.460)
Log head depth	0.82 (0.514)	1.50 (0.212)	31.46 (<0.001)	1.37 (0.319)
Log head width	1.49 (0.282)	1.32 (0.336)	40.65 (<0.001)	1.25 (0.361)
Log torso length	3.49 (0.063)	2.29 (0.132)	64.79 (<0.001)	1.87 (0.196)
Log tail length	1.17 (0.373)	0.62 (0.689)	17.02 (0.003)	0.57 (0.724)
Log tail fin depth	1.56 (0.0265)	0.44 (0.811)	15.37 (0.004)	0.53 (0.747)
Log tail muscle depth	0.35 (0.787)	0.81 (0.572)	8.47 (0.017)	0.83 (0.557)
Log tail muscle width	0.72 (0.566)	0.39 (0.846)	5.37 (0.046)	0.39 (0.844)
B) Mid				
Log head length	6.44 (0.013)	0.44 (0.809)	34.76 (<0.001)	0.32 (0.886)
Log head depth	0.98 (0.442)	0.44 (0.811)	44.14 (<0.001)	0.58 (0.718)
Log head width	0.33 (0.803)	0.70 (0.637)	95.66 (<0.001)	1.06 (0.441)
Log torso length	3.48 (0.064)	**6.09 (0.009)**	171.93 (<0.001)	**4.69 (0.022)**
Log tail length	2.44 (0.131)	3.02 (0.072)	31.44 (<0.001)	2.00 (0.173)
Log tail fin depth	12.62 (0.001)	**4.89 (0.019)**	62.59 (<0.001)	1.57 (0.262)
Log tail muscle depth	1.00 (0.436)	2.01 (0.171)	44.00 (<0.001)	1.88 (0.193)
Log tail muscle width	2.91 (0.094)	0.63 (0.686)	69.65 (<0.001)	0.54 (0.743)
C) Late				
Log head length	2.50 (0.125)	1.61 (0.252)	19.51 (0.002)	0.12 (0.984)
Log head depth	8.03 (0.007)	2.56 (0.105)	393.34 (<0.001)	1.77 (0.215)

Log head width	5.47 (0.020)	1.04 (0.450)	33.38 (<0.001)	0.17 (0.966)
Log torso length	1.87 (0.204)	0.68 (0.648)	47.59 (<0.001)	0.37 (0.859)
Log tail length	2.55 (0.121)	0.67 (0.657)	84.60 (<0.001)	0.74 (0.610)
Log tail fin depth	0.62 (0.619)	0.69 (0.644)	7.31 (0.024)	0.48 (0.785)
Log tail muscle depth	1.06 (0.411)	1.25 (0.363)	39.67 (<0.001)	0.65 (0.669)
Log tail muscle width	13.86 (0.001)	1.05 (0.445)	24.26 (<0.001)	1.09 (0.426)

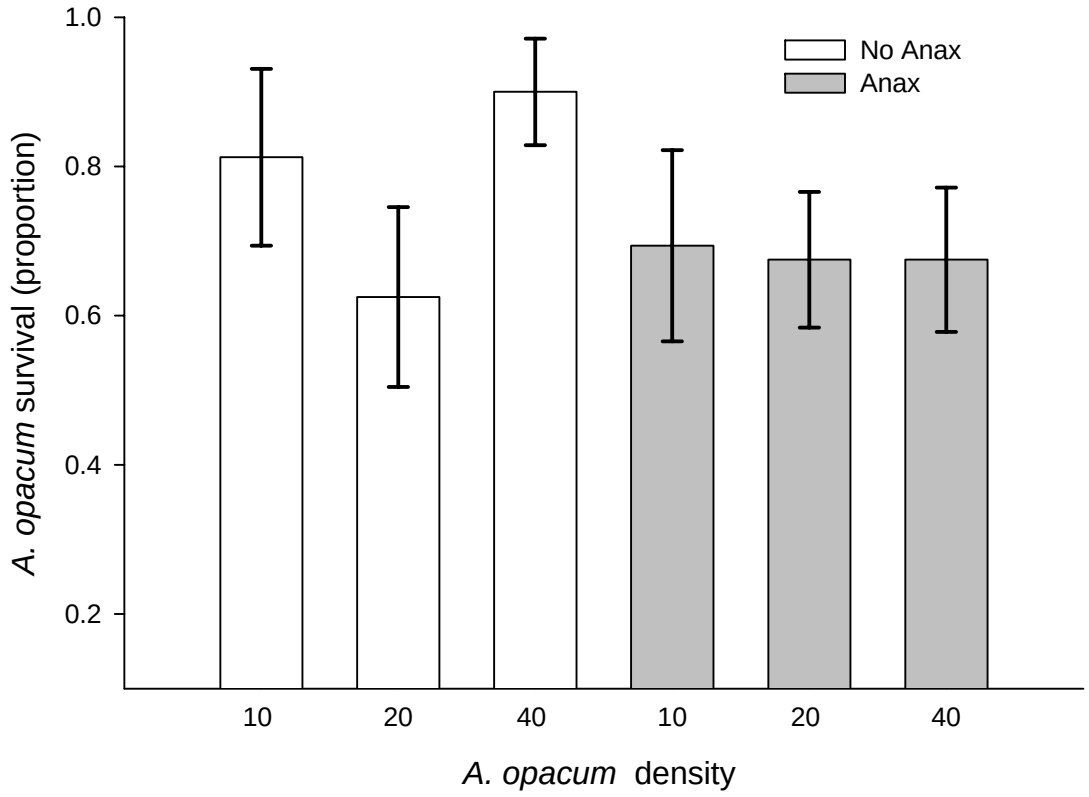


Figure 6. Survival of six *A. opacum* phenotypes in the plasticity experiment. Data are mean proportions with ± SE.

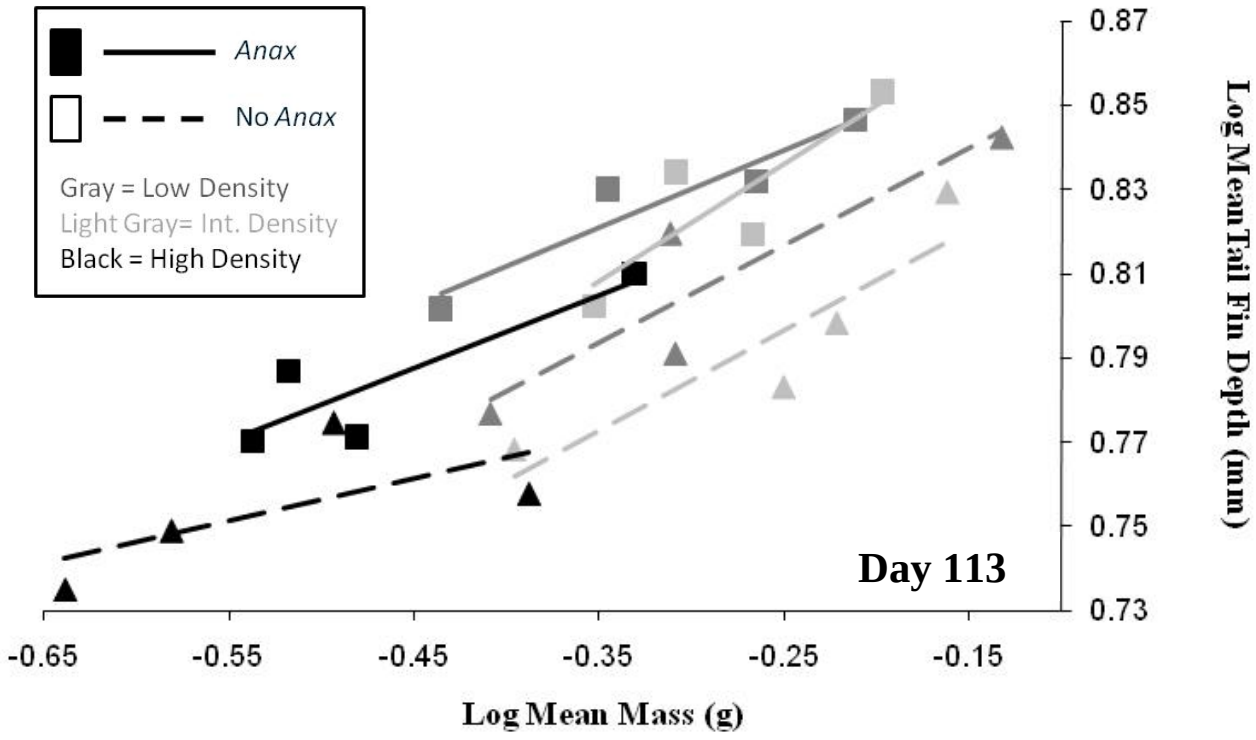


Figure 7. Morphological changes in *A. opacum* tail fin depth (corrected for *A. opacum* mass) during the middle sampling period (day 113) for *A. opacum* tail fin depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.

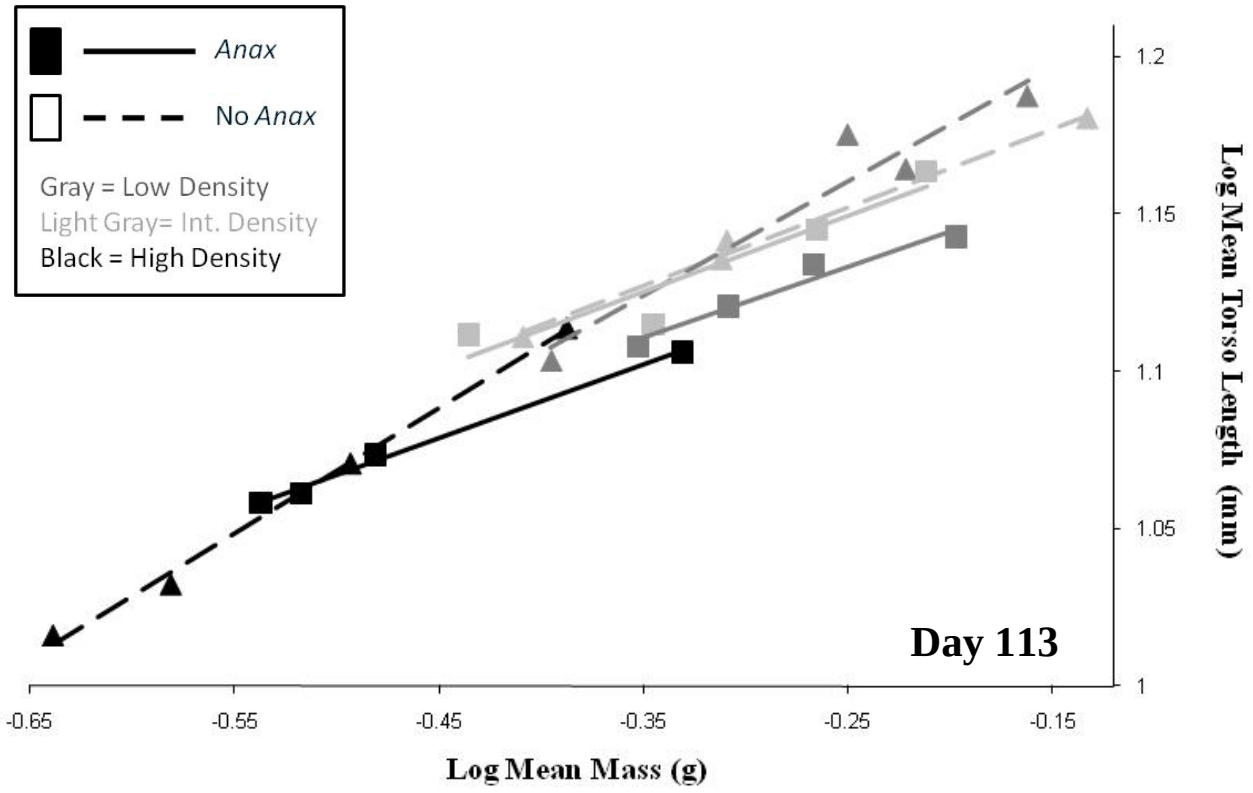


Figure 8. Morphological changes in *A. opacum* torso length (corrected for *A. opacum* mass) during the middle sampling period (day 113) for *A. opacum* torso length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.

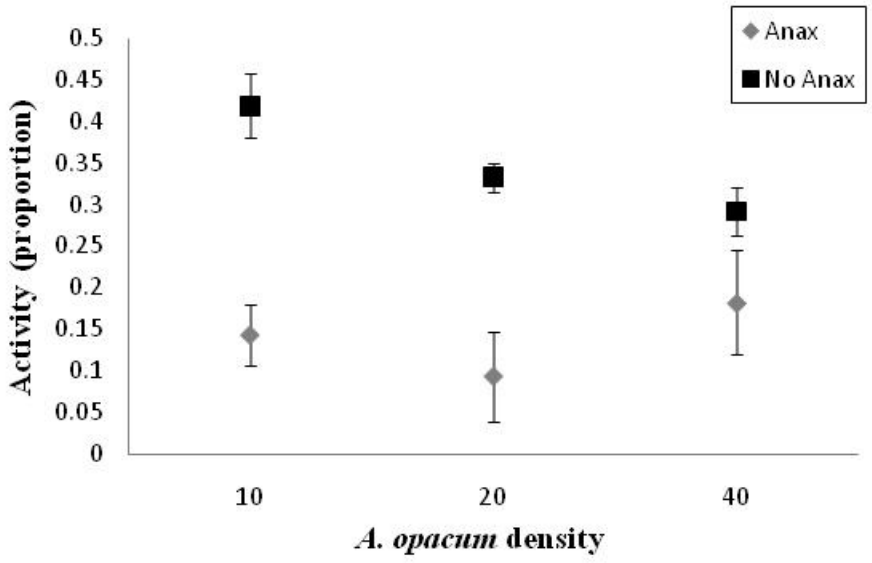


Figure 9. Behavioral responses (proportion active) of *A. opacum* in the presence of *Anax* cues (grey diamonds) and in absence of *Anax* cues (black squares). Data are means ± SE.

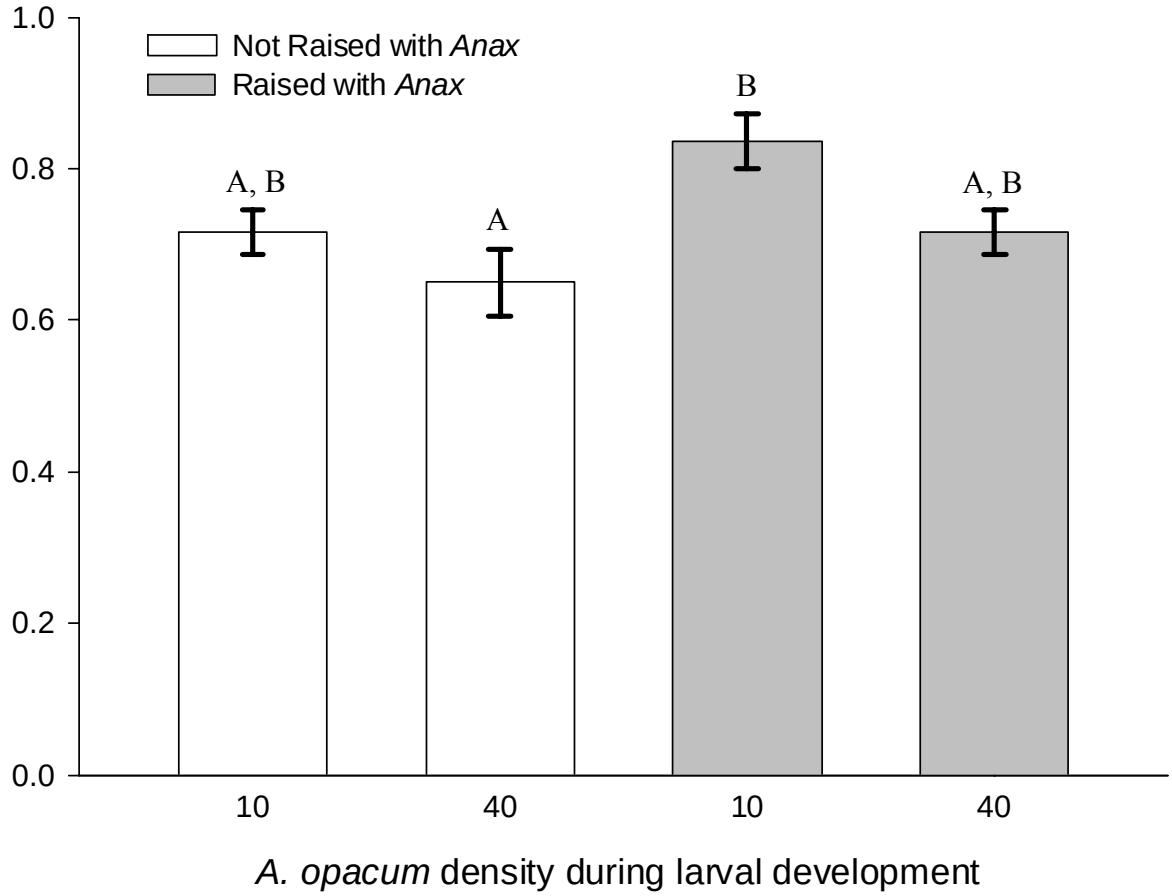


Figure 10. Survival of four *A. opacum* phenotypes in vulnerability trials with *Anax*. Data are mean proportions with ± SE. Letters above bars indicate statistical differences among means.

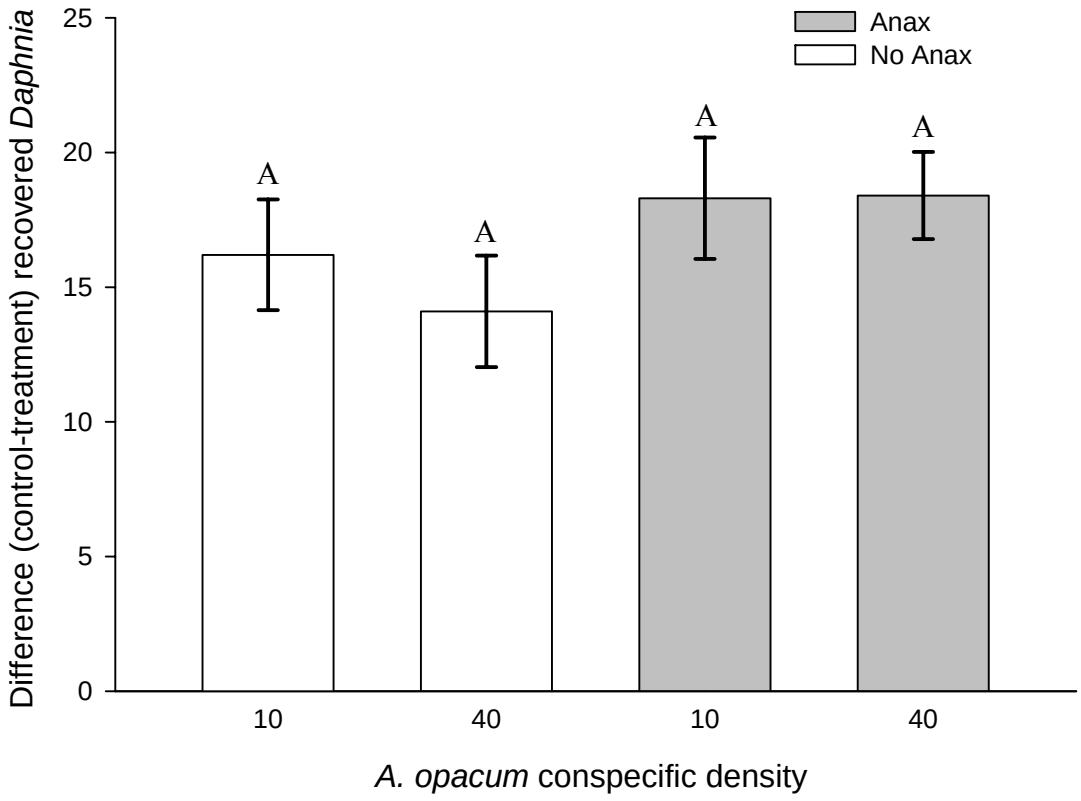


Figure 11. Mean differences of total number of *Daphnia* recovered from foraging efficiency trials with four *A. opacum* phenotypes. Data are means $\pm$ SE.

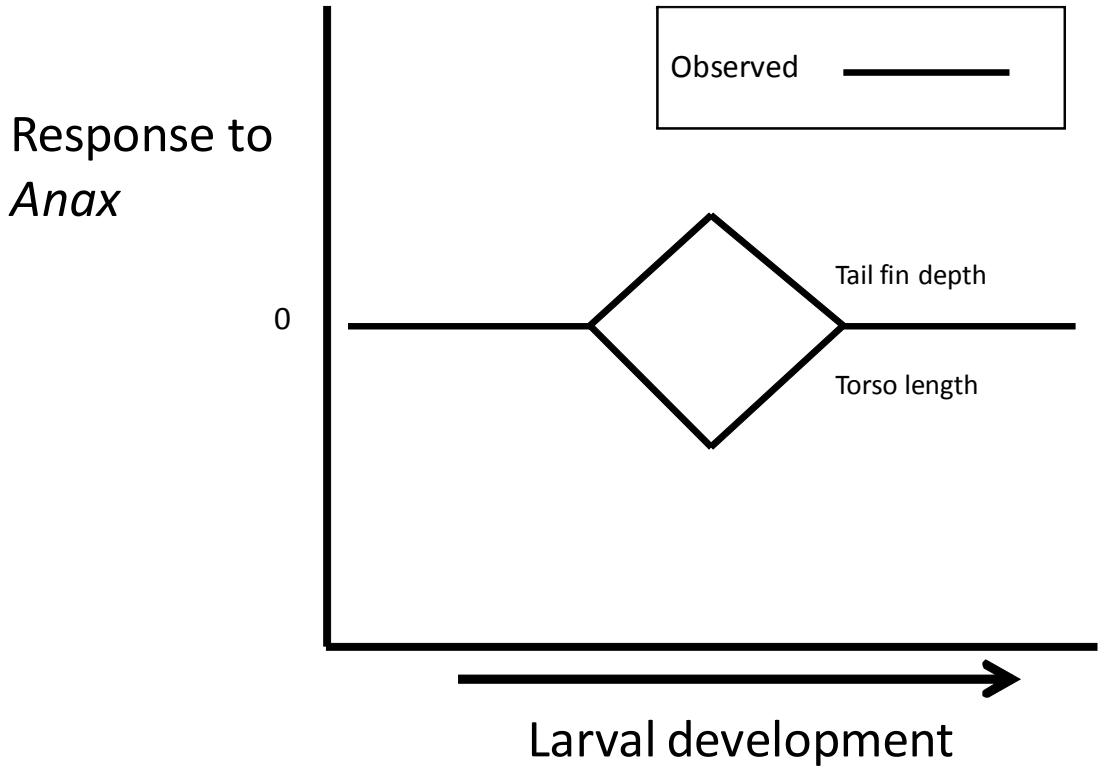


Figure 12. Summary of *A. opacum* induced morphological traits in response to *Anax* throughout *A. opacum* larval development.

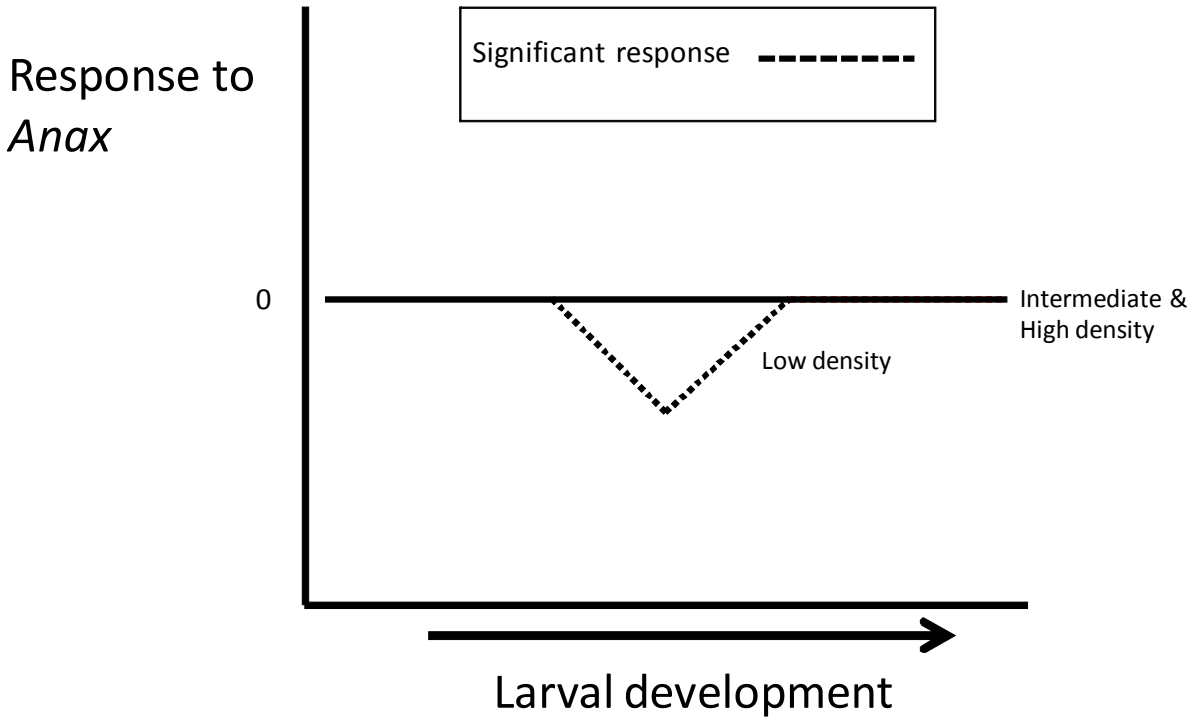


Figure 13. Summary of *A. opacum* torso length at each conspecific density in response to the presence of *Anax* throughout *A. opacum* larval development.

CHAPTER 4: The Effects of Different Forms of Habitat Complexity on the Strength of Intraguild Interactions

Introduction

Intraguild predation (IGP) is a process involving competing predator species that can also eat one another. IGP can be a driving force in regulating and structuring natural communities (Polis et al. 1989). Theory predicts that food webs with IGP are unstable relative to other types of food webs and that the strength of IGP can be so strong that it drives intermediate predator and shared prey populations to extinction in local food webs (Holt and Polis 1997, Mylius et al. 2001). Theory also predicts that intermediate predators must be superior competitors for a shared prey resource in order to persist with top predators (Holt and Polis 1997). Although intermediate predators face strong consumptive and competitive pressure, ecologists often see top and intermediate predators and their prey resources persisting together in nature (Polis and Winemiller 1996, Arim and Marquet 2004). Therefore, ecologists have begun to expand theoretical models of IGP to reconcile this discrepancy between IGP theoretical predictions and IGP empirical work (Amaresekare 2007a,b, Daugherty et al. 2007, Holt and Huxel 2007). There has also been a call for more empirical work testing the factors and theoretical predictions that influence the strength of IGP interactions since empirical data is lacking (Rosenheim 2007). One factor that may influence the strength of intraguild interactions is the amount of complexity within a habitat.

Habitat complexity broadly refers to heterogeneity in the physical structures making up an environment (Bell et al. 1991). A considerable amount of work has been done to understand the importance of habitat complexity and its subsequent effects on species diversity (Huffaker

1958, MacArthur et al. 1966, Root 1973, August 1983, Kareiva 1987, Langellotto and Denno 2004). Habitat complexity can influence species diversity by altering the strength of predator-prey interactions. The influence of habitat complexity can occur via three different mechanisms: 1) by reducing encounter rates between predators and prey through reduced perception of prey by predators (Crowder and Cooper 1982, Finke and Denno 2002, Janssen et al 2007), 2) by providing refugia for prey from predators (Rozas and Odum 1988, Persson and Eklov 1995) and 3) by enhancing foraging efficiency of predators on prey by providing additional ambush or perch sites (Fenno et al. 2002, Warfe and Barmuta 2006). Few studies have considered how predators can gain an advantage in foraging efficiency due to changes in habitat complexity in the environment (Denno et al. 2005).

Most work examining the effects of habitat complexity on predator-prey or predator-predator interactions has focused on habitat complexity as defined by one particular measure of habitat structure, the amount of one kind of vegetation present. Generally in simpler food webs, as the amount of vegetation in a terrestrial or aquatic habitat increases, fewer prey individuals are consumed by predators (Crowder et al. 1998, Hansen 2000, Denno et al. 2005). In IGP food webs, a higher amount of vegetation reduces encounters between top and intermediate predators and therefore decreases the strength of predator-predator interactions (Finke and Denno 2002, 2006). However, shared prey consumption by top and intermediate predators in IGP food webs with higher amounts of vegetation increases, since both predators are not interfering with one another (Finke and Denno 2002, 2006). Hence, one aspect of habitat complexity (e.g., the amount of vegetation) can have complex effects on IGP interactions within a food web (Finke and Denno 2002, 2006).

Most ecological communities have several different aspects of a habitat that contribute to its overall complexity. Studies on the effects of one form of habitat complexity on species interactions (e.g., competition and predation) are relatively common (McCoy and Bell 1991); however, studies that incorporate the effects of multiple measures of habitat complexity on species interactions are not. Habitat complexity can be measured by multiple indices independently to fully capture the different aspects of habitat structure as shown by Beck (2000). In one of the few studies to consider multiple aspects of habitat complexity, Beck (2000) found that density and surface area of rocks in a simulated habitat had differing effects on gastropod assemblages from rocky intertidal shores. Despite the accumulation of evidence for the effects of habitat complexity on predator-prey interactions, there is a paucity of data detailing how different forms of habitat complexity influence the strength of predator-prey interactions. Ecologists are now beginning to focus on other aspects of habitat complexity and the subsequent effects on species interactions.

The importance of distinguishing different aspects of habitat complexity when evaluating predator-prey interactions can be especially important if different taxa respond to different aspects of habitat complexity differently and the particular response of taxa depends on particular species traits. For example, differences in predator foraging strategy may cause different predator species to respond differently to a particular form of habitat complexity (Formanowicz 1982, Swisher et al. 1998, Lancaster and Mole 1999, Hughes and Grabowski 2006). A predator with a sit-and-pursue foraging strategy may increase foraging efficiency in complex habitats that provide them with access to additional perch or ambush sites (such as utilizing high amounts of emergent vegetation to move up or down in the water column) (James and Heck 1994). Recent experimental work by Warfe and Barmuta (2004, 2006) supports this

idea, showing that fish predators likely switched from searching for prey to ambushing prey in the most structurally complex environments to maintain high prey capture efficiency. In contrast, active foraging predators in complex habitats (such as high amounts of emergent vegetation) may experience a reduction in foraging efficiency due to reduced perception of prey and encounter rates with prey (Savino and Stein 1989).

Prey also respond to habitat complexity and have been known to use habitat complexity as a refuge to hide from predators or to reduce encounter rates with predators (Persson and Eklov 1995, Denno et al. 2005). The particular response of prey, however, may also be dependent on the particular form of habitat complexity in the environment. Prey species have been known to prefer certain refuges over others since one refuge provides more hiding space (Lima and Dill 1990). Preference for one form of complexity could also be to avoid particular forms of habitat complexity that may enhance encounter and mortality rates with predators (Denno et al. 2005). Therefore, there is potential for different forms of habitat complexity (e.g., amount of emergent vegetation versus amount of benthic leaf litter in aquatic habitats) to have strong impacts on the strength of predator-prey interactions by creating a mosaic of heterogeneity in the environment with advantages and disadvantages for both predators and prey.

To address the effects of two different measures of habitat complexity on IGP interactions, I conducted an experiment in mesocosms to examine how the main and interactive effects of two different kinds (amount of emergent aquatic vegetation versus amount of benthic leaf litter) of habitat complexity influences the effect of a sit-and-pursue top predator (larval dragonflies; *Anax* spp.) on fitness components of an active-foraging intermediate predator (larval salamanders; *Ambystoma opacum*). I expected increasing amounts of emergent vegetation to enhance the effect of *Anax* in reducing *A. opacum* fitness by providing *Anax* with perch sites and

thus increasing encounter rates. I also expected that increasing amounts of leaf litter would reduce the effect of *Anax* on *A. opacum* fitness by providing *A. opacum* with benthic refuges to hide from *Anax*.

Methods

Larval salamanders, *Ambystoma opacum*, are one of the common salamander predators of temporary ponds in the eastern United States. *A. opacum* can function as keystone predators of larval anurans (Morin 1995, Chalcraft & Resetarits 2003a) and are superior competitors compared to two other salamander species that arrive later in fishless ponds (Stenhouse et al. 1983, Boone et al. 2002). When present, however, larval aeshnid dragonflies (primarily *Anax* spp.) are one of the top invertebrate predators in fishless ponds (Van Buskirk 1988, Wilbur & Fauth 1990). Larval *Anax* are typically considered sit-and-pursue predators (Pritchard 1965). *Anax* consumes *A. opacum* and both predators consume a common prey resource (macroinvertebrates and larval anurans) during the spring (e.g., *Bufo* spp., *Pseudacris* spp.), however, *Anax* does not consume as much zooplankton as *A. opacum* in laboratory trials (Davenport, unpublished data). *A. opacum* are unable to eat some of the larger prey (e.g., overwintered *Rana* spp. tadpoles) that *Anax* can eat due to the fact that *A. opacum* consumes food whole thereby gape size prevents them from eating larger prey items (Smith 1990, Morin 1995, Chalcraft and Resetarits 2003b). Both predator species have been found together in ponds of varying habitat complexity (the amount of emergent vegetation and amount of leaf litter) in eastern NC.

I designed a fully factorial experiment where I manipulated three factors; the amount of emergent vegetation (*Myriophyllum* spp.; absent, low (15 stems), or high (30 stems)), the amount

of hardwood leaf litter (low (500 grams) or high (1.5 kilograms)), and the presence of *Anax* (0 or 1). This combination of factors yielded 12 treatments which were replicated once in each of four spatial blocks of mesocosms. The 48 mesocosms, modified 1000 L cattle tanks, were located at the West Research Campus of East Carolina University, Pitt County, NC. Mesocosms represent an important technique to study the ecology of larval amphibians because they allow the experimenter to create many identical and independent experimental units to which they can apply particular treatments of interest (Morin 1989, Wilbur 1989). Fiberglass mesh screens were placed on the ponds to prevent colonization by unwanted organisms and escape of experimental organisms during the study. Ponds were equipped with PVC standpipes that allowed me to drain water levels in tanks in accordance with a natural pond hydroperiod (187 days; see Wilbur 1987 for drying curve). This pond hydroperiod was realistic based on prior literature and field observations on the wide range of pond hydroperiods in eastern NC (Davenport, pers. obs.).

All animals were collected from the Croatan National Forest (74 km from Greenville, NC) unless otherwise noted. All mesocosms were filled with well water (13-14 November 2008) and received a standard aliquot of plankton from local ponds (1 December 2008), 9 overwintered *Rana sphenocephala* tadpoles (7 December 2008) and 220 *Bufo terrestris* tadpoles (7 April 2009 from local Greenville, NC ponds) to serve as prey to both predators *A. opacum* clutches were collected from the Croatan National Forest from 2-25 November 2008 and kept in the lab until 2 December 2008. On 2 December 2008, I commenced hatching of *A. opacum* by flooding the eggs with filtered pond water (Petranka et al. 1982). Larval *A. opacum* were counted from clutches (with equal representation from 19 clutches), randomly assigned to one of the 48 mesocosms, and then placed into mesocosms on 10 December 2008.

I randomly assigned one of the twelve treatments described above to a mesocosm within each of four spatial blocks. All tanks received leaf litter (either 500 g or 1.5 kg depending on the treatment) that was collected from the Otter Creek Natural Area, Pitt Co., NC (predominantly *Fagus grandifolia*, American Beech). Leaf litter was mixed, weighed out in buckets and distributed to mesocosms on 24 November 2008. *Myriophyllum* spp., an aquatic macrophytes, was collected from natural ponds in the Croatan National Forest on 2 December 2008. *Myriophyllum* stems were washed and planted (either a low number, 15 stems or high number, 30 stems depending on the treatment) in the assigned mesocosms on 3-4 December 2008. Each stem was planted in a single plastic cup filled with sterilized play sand. *Anax* larvae were collected from the Croatan National Forest, weighed and randomly assigned to mesocosms receiving *Anax* on 12 December 2008. Thus all organisms available at the time were assigned to mesocosms by 12 December 2008 and the experiment was initiated. All ponds were monitored daily for the emergence of metamorphosing individuals. The experiment ended 5-7 June 2009 when water levels in mesocosms reached a depth of 5 cm which can be fatal due to heat stress. Any remaining larvae likely would not complete metamorphosis before mesocosms completely dried.

A. opacum performance in each mesocosm was characterized by the mesocosm average for three response variables: survival, mass at metamorphosis and larval period. Survival was defined as the mean proportion of salamanders that metamorphosed from a particular mesocosm. Mass at metamorphosis was represented by the mean mass of all salamanders that successfully metamorphosed (individuals which had completely absorbed their gills) from a mesocosm. Larval period was represented by the average time it took salamanders to complete metamorphosis in a particular mesocosm. All of these response variables have been repeatedly

shown to have important consequences for the individual fitness of an adult amphibian, the persistence of amphibian populations (Semlitsch et al. 1988, Berven 1990, Scott 1994) and population regulation of *A. opacum* populations (Taylor & Scott 1997, Taylor et al. 2006). Survival data were natural log transformed to provide a measure of instantaneous per capita mortality rates. I added 0.01 to the proportion surviving in a mesocosm survival in some mesocosms was 0 (the log of 0 is undefined).

A factorial ANOVA was conducted on each of the three *A. opacum* response variables in mesocosms without *Anax* to elucidate the main and interactive effects of two different types of habitat complexity (across all combinations of the amount of complexity) on *A. opacum* performance. Survival was low in treatments with *Anax*. No salamanders survived in environments with high amounts of leaf litter and with high amounts of *Myriophyllum* with *Anax* present. Therefore, some treatments were not equally replicated thus I lacked statistical power to conduct a three-way ANOVA to reveal the main and interactive effects of *Anax* and both kinds of habitat complexity. Subsequently, I estimated the impact of *Anax* on *A. opacum* performance in each of the six environments with a log response ratio (effect size; Hedges et al. 1999). The log response ratio (effect of *Anax*) in each kind of environment was derived for each spatial block by taking the log of the ratio of the response in a particular environment (treatment) where *Anax* was present and the response in the same environment within that block where *Anax* was absent. I then conducted a factorial ANOVA for the main and interactive effects of habitat complexity (leaf litter and *Myriophyllum*) on the impact of *Anax* for each of the three *A. opacum* response variables.

Results

Increasing the amount of leaf litter in a pond lowered *A. opacum* survival when *Anax* was not present ($F_{1,18}$ = 14.65, P = 0.001; Figure 14). Neither the amount of *Myriophyllum* ($F_{2,18}$ = 0.99, P = 0.392; Fig. 14) or the interaction between amount of leaf litter and the amount of *Myriophyllum* ($F_{2, 18}$ = 1.55, P = 0.239; Fig. 14) affected *A. opacum* survival in ponds with no *Anax*. *A. opacum* mass at metamorphosis was always significantly higher in high leaf litter environments than in low leaf litter environments ($F_{1,18}$ = 14.13, P = 0.001; Fig. 15) while the amount of *Myriophyllum* had marginally significant effects ($F_{2, 18}$ = 3.35, P = 0.058; Fig. 15) on *A. opacum* mass at metamorphosis. The effect of leaf litter amount on *A. opacum* mass at metamorphosis appeared to be dependent on the amount of *Myriophyllum* present in the environment ($F_{2,18}$ = 3.43, P = 0.055; Fig. 15) but this interaction appears to be driven by one treatment (high leaf litter and low *Myriophyllum*). Individuals in high leaf litter and low *Myriophyllum* environments had the greatest mass at metamorphosis relative to the other five treatments. Larval period was not affected by the amount of leaf litter ($F_{1,18}$ = 0.05, P = 0.834; Fig. 16), the amount of *Myriophyllum* ($F_{2, 18}$ = 0.32, P = 0.729; Fig. 16) or the interactive effects of leaf litter amount and *Myriophyllum* amount ($F_{2, 18}$ = 1.08, P = 0.361; Fig. 16).

A. opacum survival was reduced as more *Myriophyllum* was present in the environment when *Anax* was present ($F_{2,18}$ = 17.86, P < 0.001; Fig. 17). However, the impact of *Anax* on *A. opacum* survival was not affected by the amount of leaf litter in the environment ($F_{1, 18}$ = 1.18, P = 0. 291; Fig. 17). The influence of *Myriophyllum* on the impact that *Anax* had on *A. opacum* survival depended on the amount of leaf litter in the environment ($F_{2, 18}$ = 6.23, P = 0.009; Fig. 17). This effect was likely driven by the positive effect of *Anax* on *A. opacum* survival in environments with low amounts of *Myriophyllum* and high amounts of leaf litter (Fig. 17).

Leaf litter affected the impact of *Anax* on *A. opacum* mass at metamorphosis ($F_{1, 9} = 7.61$, $P = 0.022$; Fig. 18). The impact of *Anax* on *A. opacum* mass at metamorphosis was dependent on the amount of leaf litter and the amount of *Myriophyllum* present in a pond ($F_{1, 9} = 6.11$, $P = 0.035$; Fig. 18). Specifically, this pattern appears to be driven by the negative impact of *Anax* on *A. opacum* mass in the treatment with a high amount of leaf litter but no *Myriophyllum* present (Fig. 18). The amount of *Myriophyllum* present did not influence the impact of *Anax* on *A. opacum* mass at metamorphosis ($F_{2, 9} = 2.86$, $P = 0.109$; Fig. 18). The impact of *Anax* on *A. opacum* larval period was not affected by: the amount of leaf litter ($F_{1,9} = 0.27$, $P = 0.614$), the amount of *Myriophyllum* ($F_{2, 9} = 0.52$, $P = 0.612$) or the interactive effects of leaf litter amount and *Myriophyllum* amount ($F_{1, 9} < 0.01$, $P = 0.989$).

Discussion

By comparing the responses of top and intermediate predators to two different forms of habitat complexity, I was able to establish that multiple metrics of habitat complexity can have complex and even unexpected effects on intermediate predator performance. Increasing levels of one form of habitat complexity, leaf litter amount, was hypothesized to be beneficial for intermediate predators by providing refugia from top predators. Surprisingly, I instead found that increasing amounts of leaf litter without top predators led to a decrease in survival of intermediate predators. As a result of the high mortality associated with high leaf litter environments, the largest intermediate predators metamorphosed from high leaf litter environments. This is due to high amounts of leaf litter producing a "thinning" effect (like top predators in chapter 2) on *A. opacum* mass whereby leaf litter indirectly benefits surviving *A. opacum* by reducing the number of *A. opacum* that survivors must compete with for food. Larval

period of intermediate predators was not significantly affected by the form or amount of habitat complexity. As predicted, increasing the amount of *Myriophyllum* in the environment increased the negative impact of top predators on intermediate predator survival. This highlights the importance of form and amount of complexity. Specifically, how one form of habitat complexity can affect intermediate performance and another form of habitat complexity can affect the strength of intraguild interactions in surprising ways.

I did not expect either kind of habitat complexity (leaf litter amount or *Myriophyllum* amount) to have a detrimental effect on *A. opacum* growth or survival without *Anax* present. However, *A. opacum* survival was severely reduced in high leaf litter environments (Fig. 14). Other researchers have recently found that the composition of leaf litter input can alter the performance of larval amphibians (Rubbo and Kiesecker 2004, Williams et al. 2008, Stoler and Relyea in press). I have two interrelated hypotheses that could explain the underlying mechanism for this effect of leaf litter amount on *A. opacum* survival.

The first hypothesized mechanism in which leaf litter amount could have negatively affected *A. opacum* survival is by leaching compounds into the water, thereby changing water chemistry. Recent work has shown that beech leaves can enhance water pH relative to that observed in ponds with other species of broadleaf litter (Stoler and Relyea in press). Interestingly, some salamander species (*A. tigrinum*) become less efficient in foraging when water pH is lower (Kiesecker 1996). *A. tigrinum* embryos experience high mortality in water with low pHs and adult *A. tigrinum* avoid waters with a low pH (Whiteman et al. 1995, Lannoo 2005). It is possible that *A. opacum* larvae are the opposite of *A. tigrinum* larvae and have a lower predatory success in ponds with higher pHs. *A. opacum* are primarily found in North Carolina ponds with a median pH of 5.8 (Smith and Braswell 1994). Ponds with high pHs could

be detrimental to *A. opacum* development and foraging success during their larval period. Therefore, one hypothesis could be that *A. opacum* larvae perform better in ponds with low pHs than in ponds with high pHs in nature.

The second hypothesized mechanism in which leaf litter amount could have affected *A. opacum* survival is indirectly by decreasing the abundance of zooplankton (prey for *A. opacum*). Beech litter has been found to cause the abundance of zooplankton to be lower than that observed in ponds with other species of hardwood litter (Stoler and Relyea in press). The direct effect of beech leaves on zooplankton abundance may have been further magnified by the amount of beech leaves placed into mesocosms for this study. Further evidence from another study has shown that one type of zooplankton, cladocerans, was at lowest abundance in mesocosms with 900 g of mixed deciduous leaf litter vs. 100 g or 300 g of mixed deciduous leaf litter (Rubbo et al. 2008). Since cladoceran abundance was not statistically different among treatments, the authors did not further discuss these trends in cladoceran abundance. Nonetheless, the amount and type of leaf litter in an environment can differentially impact the performance of organisms at lower trophic levels and potentially destabilize food webs (Rubbo and Kiescker 2004).

The results presented here are surprising given that *A. opacum* are commonly found in ponds with hardwood leaf litter (Petranka 1998). One possible reason that leaf litter amount may have affected *A. opacum* survival is that natural ponds usually vary in leaf litter composition. This study may have magnified the adverse effects of beech leaves by only using beech leaves. Mixed broadleaf treatments had higher zooplankton abundances and lower pHs in comparison to beech leaf only treatments (Relyea and Stoler in press). These recent studies on leaf litter input provide some support for my hypotheses regarding the mechanisms driving the adverse effects of

leaf litter on performance of *A. opacum* (Rubbo and Kiesecker 2004, Williams et al. 2008, Rubbo et al. 2008, Stoler and Relyea in press).

One of the most interesting results from this experiment is that one measure of habitat complexity, amount of leaf litter, did not benefit intermediate predators in mesocosms with top predators as expected. This hypothesis was originally based on two mechanisms that were found to be important in other studies: 1) increased refuge use by prey and 2) reduced foraging efficiency of top predators in complex environments. Unfortunately, I did not make behavioral observations during this study. However, in aquatic systems, ambystomatid salamanders reduce activity levels in the presence of predators and increase refugia use when predators are present (Kats et al. 1988, Huang and Sih 1990, Yurewicz 2004, Davenport, chapter 3). Hossie and Murray (2010) have also shown that in environments with high amounts of leaf litter, fewer tadpoles were consumed and handling times were longer for larval aeshnid dragonflies. One benefit of high amounts of leaf litter was an increase in *A. opacum* mass at metamorphosis in comparison to *A. opacum* mass at metamorphosis in low leaf litter environments. High amounts of leaf litter severely reduced the survival of *A. opacum* which further reduced encounter rates with *Anax* and left the few survivors in those environments with more resources to grow larger (Figs. 14-15). This suggests that the effect of top predators on intermediate predator mass was beneficial for the surviving intermediate predators since leaf litter amount alone reduced on intermediate predator survival.

Top predators benefited from increasing the amount of emergent vegetation in the environment regardless of leaf litter amount (Fig. 17). With the exception of the high leaf litter and low *Myriophyllum* environment, the impact of *Anax* on *A. opacum* survival was as originally predicted. *Anax* were observed using the *Myriophyllum* stems as perch sites during the

experiment supporting the hypothesis that *Anax* likely increased use of *Myriophyllum* perches as more became available (Davenport, personal observation). Although larval dragonflies rely on highly developed vision to detect prey, the increase in *Myriophyllum* did not decrease prey consumption as seen in other studies (Folsom and Collins 1984, Babbitt and Jordan 1996, Tarr and Babbitt 2002; but see Michel and Adams 2009). The impact of *Anax* on *A. opacum* survival in high leaf litter and low *Myriophyllum* environments did not follow the expected pattern. This pattern was likely driven by reduced survival from leaf litter alone therefore causing fewer *A. opacum* to encounter *Anax* and be consumed. It is important to note that this experiment differed from findings of previous work in that prey typically have greater survival in the most complex environments with predators (Denno et al. 2005). No intermediate predators survived with top predators in the most complex environments (high leaf litter amounts and high *Myriophyllum*) of this experiment (Fig. 17). This negative effect on *A. opacum* survival is likely driven by the strong additive effects of leaf litter amount and the beneficial increase in *Anax* capture rates from *Myriophyllum* perches.

This study illustrates the importance of examining the main and interactive effects of multiple measures of habitat complexity on the strength of IGP interactions. One metric of habitat complexity (leaf litter amount) alone can indirectly reduce intermediate predator survival in an unexpected manner (e.g., by directly reducing the availability of prey resources to intermediate predators) but another metric (emergent vegetation amount) also reduces intermediate predator survival by increasing foraging efficiency of top predators. Although I have no evidence of a shift in foraging mode of top predators, work by Michel and Adams (2009) suggests that high habitat complexity can lead to a shift in foraging mode and behavior of invertebrate predators. This shift led to a stronger consumptive effect of predators on prey items.

Both measures (high amounts of leaf litter and high amounts of emergent vegetation) decrease the likelihood of persistence between top and intermediate predator. Therefore, intermediate predators may also change their behavior and avoid certain forms of habitat complexity (ones that enhance mortality risk) and preferentially choose to stay near other forms of habitat complexity (ones that reduce mortality risk). This has significant implications for food webs with IGP interactions and potential discrepancies with IGP theory and empirical data.

Further investigation is warranted of additional metrics of habitat complexity to fully understand the effects of habitat complexity on predator behavioral shifts, species interactions and the structuring of biological communities. My study suggests that habitat complexity can increase the strength of IGP interactions between top and intermediate predators in environments and lead to destabilization of IGP food webs. Theoretical work incorporating multiple metrics of habitat complexity and its effects on species persistence needs to be developed in the same manner as food web modules have recently been (Daugherty et al. 2007, Holt and Huxel 2007). Ecologists are aware that natural communities are often far more complex than what can be mimicked in experimental settings. Expanded theory would provide biologists with a stronger foundation to build upon since simple models may not capture the intricacy of the natural world. This would allow ecologists to understand the full complement of species interactions within a food web in light of habitat complexity that differs in more than one measure. Future studies should consider multiple measures of habitat complexity in order to encapsulate the full spectrum of structure that could be contributing to species persistence.

References

Arim, M. and P. Marquet. 2004. Intraguild predation: a widespread interaction related to species biology. Ecology Letters 7:557-564.

Babbitt, K.J. and F. Jordan. 1996. Predation on *Bufo terrestris* tadpoles: effects of cover and predator identity. Copeia 1996:485-488.

Bell, S.S., E.D. McCoy, and H.R. Mushinsky, editors. 1991. Habitat structure: the physical arrangement of objects in space. Chapman and Hall, New York, New York, USA.

Beck, M.W. 2000. Separating the elements of habitat structure: independent effects of habitat complexity and structural components on rocky intertidal gastropods. Journal of Experimental Marine Biology and Ecology 249:29-49.

Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71:1599-1608.

Boone, M.D., D.E. Scott, and P.N. Niewiarowski. 2002. Effects of hatching time for larval ambystomatid salamanders. Copeia 2002:511-517.

Chalcraft, D.R. and W.J. Resetarits, Jr. 2003a. Predator identity and ecological impacts: functional redundancy or functional diversity? Ecology 84:2407-2418.

Chalcraft, D.R. and W.J. Resetarits, Jr. 2003b. Mapping functional similarity among top predators on the basis of trait similarities. American Naturalist 162:390-402.

Crowder, L.B. and W.E. Cooper. 1982. Habitat structural complexity and the interaction between bluegills and their prey. Ecology 63:1802-1813.

Crowder, L.B., E.W. McCollum, and T.H. Martin. 1998. Changing perspectives on food web interactions in lake littoral zones. Pages 240-249 *in* E. Jeppesen, M. Sondergaard, M.

Sondergaard, and K. Christoffersen, editors. The structuring roles of submerged macrophytes in lakes. Springer, New York, New York, USA.

Daugherty, M.P., J.P. Harmon, and C.J. Briggs. 2007. Trophic supplements to intraguild predation. Oikos 116:662-677.

Denno, R.F., C. Gratton, M.A. Peterson, G.A. Langellotto, D.L. Finke, and A.F. Huberty. 2002. Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. Ecology 83:1443-1458.

Denno, R.F., D.L. Finke, and G.A. Langellotto. 2005. Direct and indirect effects of vegetation structure and habitat complexity on predator-prey and predator-predator interactions. Pages 211-239 *in* P. Barbosa and I. Castellanos, editors. Ecology of predator-prey interactions. Oxford University Press, Oxford, UK.

Finke, D.L. and R.F. Denno. 2002. Intraguild predation diminished in complex-structured vegetation: implications for prey suppression. Ecology 83:643-652.

Finke, D.L. and R.F. Denno. 2006. Spatial refuge from intraguild predation: implications for prey suppression and trophic cascades. Oecologia 149:265-275.

Folsom, T.C. and N.C. Collins. 1984. The diet and foraging behavior of the larval dragonfly *Anax junius* (Aeshnidae), with an assessment of the role of refuges and prey activity. Oikos 42:105-113.

Formanowicz, D.R., Jr. 1982. Foraging tactics of larvae of *Dytiscus verticalis* (Coleoptera: Dytiscidae): the assessment of prey density. Journal of Animal Ecology 51:757-767.

Grabowski, J.H., A.R. Hughes, and D.L. Kimbro. 2008. Habitat complexity influences cascading effects of multiple predators. Ecology 89:3413-3422.

Hansen, R.A. 2000. Effects of habitat complexity and composition on a diverse litter microarthropod assemblage. Ecology 81:1120-1132.

Hedges, L.V., J. Gurevitch, and P.S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80:1150-1156.

Holt, R.D. and G.A. Polis. 1997. A theoretical framework for intraguild predation. American Naturalist 149:745-764.

Holt, R.D. and G.R. Huxel. 2007. Alternative prey and the dynamics of intraguild predation: theoretical perspectives. Ecology 88:2706-2712.

Hossie, T.J. and D.L. Murray. 2010. You can't run but you can hide: refuge use in frog tadpoles elicits density-dependent predation by dragonfly larvae. Oecologia 163:395-404.

Huang, C. and A. Sih. 1990. Experimental studies of behaviorally mediated indirect interactions through a shared predator. Ecology 71:1515-1522.

Huffaker, C.B. 1958. Experimental studies on predation: dispersion factors and predator-prey oscillations. Hilgardia 27:343-383.

Hughes, A.R. and J.H. Grabowski. 2006. Habitat context influences predator interference interactions and the strength of resource partitioning. Oecologia 149:256-264.

James, P.L. and K.L. Heck, Jr. 1994. The effects of habitat complexity and light intensity on ambush predation within a simulated seagrass habitat. Journal of Experimental Marine Biology and Ecology 176:187-200.

Janssen, A. M.W. Sabelis, S. Magalhães, M. Montserrat, and T. Van Der Hammen. 2007. Habitat structure affects intraguild predation. Ecology 88:2713-2719.

Kareiva, P. 1987. Habitat fragmentation and the stability of predator-prey interactions. Nature 326:388-390.

Kats, L.B., J.W. Petranka, and A. Sih. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. Ecology 69:1865-1870.

Kiesecker, J. 1996. pH-mediated predator-prey interactions between *Ambystoma tigrinum* and *Pseudacris triseriata*. Ecologial Applications 6:1325-1331.

Lancaster, J. and A. Mole. 1999. Interactive effects of near-bed flow and substratum texture on the microdistribution of lotic macroinvertebrates. Archives of Hydrobiology 146:83-100.

Langellotto, G.A. and R.F. Denno. 2004. Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. Oecologia 139:1-10.

Lannoo, M. 2005. Amphibian declines: the conservation status of United States species. University of California Press, Berkeley, CA, USA.

Lima, S.L. and L.M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68:619-640.

McCoy, E.D. and S.S. Bell. 1991. Habitat structure: the evolution and diversification of a complex topic. Pages 3-27 *in* S.S. Bell, E.D. McCoy and H.R. Mushinsky, editors. Habitat structure: the physical arrangement of objects in space. Chapman and Hall, New York, New York, USA.

Michel, M.J. and M.M. Adams. 2009. Differential effects of structural complexity on predator foraging behavior. Behavioral Ecology 20:313-317.

Morin, P.J. 1989. New directions in amphibian community ecology. Herpetologica 45:124-128.

Morin, P.J. 1995. Functional redundancy, non-additive interactions, and supply-side dynamics in experimental pond communities. Ecology 76:133-149.

Mylius, S.D., K. Klumpers, A.M. De Roos, and L. Persson. 2001. Impact of omnivory and stage structure on food web composition along a productivity gradient. American Naturalist 158:259-276.

Persson, L. and P. Eklöv. 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. Ecology 76:70-81.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C., USA.

Petranka, J.W., J.J. Just, and E.C. Crawford. 1982. Hatching of amphibian embryos: the physiological trigger. Science 4556:257-259.

Polis, G.A. and K.O. Winemiller, editors. 1996. Food webs: integration of patterns and dynamics. Chapman and Hall, New York, New York, USA.

Polis, G.A., C.A. Myers, and R.D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297-330.

Pritchard, G. 1965. Prey capture by dragonfly larvae (Odonata: Anisoptera). Canadian Journal of Zoology 43:271-289.

Root, R.B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards. Ecological Monographs 43:95-124.

Rosenheim, J.A. 2007. Intraguild predation: new theoretical and empirical perspectives. Ecology 88:2679-2680.

Rozas, L.P. and W.E. Odum. 1988. Occupation of submerged aquatic vegetation by fishes: testing the roles of food and refuge. Oecologia 77:101-106.

Rubbo, M.J. and J.M. Kiesecker. 2004. Leaf litter composition and community structure: translating regional species changes into local dynamics. Ecology 85:2519-2525.

Rubbo, M.J., L.K. Belden, and J.M. Kiesecker. 2008. Differential responses of aquatic consumers to variations in leaf-litter inputs. Hydrobiologia 605:37-44

Scott, D.E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology 75:1383-1396.

Semlitsch, R.D., D.E. Scott, and J.H.K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184-192.

Smith, C.K. 1990. Effects of variation in body size on intraspecific competition among larval salamanders. Ecology 71:1777-1788.

Smith, S.D. and A.L. Braswell. 1994. Preliminary investigation of acidity in ephemeral wetlands and the relationship to amphibian usage in North Carolina. Unpublished report to the North Carolina Wildlife Resources Commision.

Stenhouse, S.L., N.G. Hairston, and A.E. Cobey. 1983. Predation and competition in *Ambystoma* larvae: field and laboratory experiments. Journal of Herpetology 17:210-220.

Stoler, A.B. and R.A. Relyea. *In press.* Living in the litter: the influence of tree leaf litter on wetland communities. Oikos.

Swisher, B.J., D.A. Soluk, and D.H. Wahl. 1998. Non-additive predation in littoral habitats: influences of habitat complexity. Oikos 81:30-37.

Tarr, T.L. and K.J. Babbitt. 2002. Effects of habitat complexity and predator identity on predation of *Rana clamitans* larvae. Amphibia-Reptilia 23:13-20.

Taylor, B.E., and D.E. Scott. 1997. Effects of larval density dependence on population dynamics of *Ambystoma opacum*. Herpetologica 53:132-145.

Taylor, B.E., D.E. Scott, and J.W. Gibbons. 2006. Catastrophic reproductive failure, terrestrial survival, and persistence of the marbled salamander. Conservation Biology 20:1457-1465.

Warfe, D.M. and L.A. Barmuta. 2004. Habitat structural complexity mediates the foraging success of multiple predator species. Oecologia 141:171-178.

Warfe, D.M. and L.A. Barmuta. 2006. Habitat structural complexity mediates food web dynamics in a freshwater macrophytes community. Oecologica 150:141-154.

Whiteman, H.H., R.D. Howard, and K.A. Whitten. 1995. Effects of pH on embryo tolerance and adult behavior in the tiger salamander, *Ambystoma tigrinum tigrinum*. Canadian Journal of Zoology 73:1529-1537.

Wilbur, H.M. 1987. Regulation of structure in complex systems: Experimental temporary pond communities. Ecology 68:1437-1452.

Wilbur, H.M. 1989. In defense of tanks. Herpetologica 45:122-123.

Wilbur, H.M. and J.E. Fauth. 1990. Experimental aquatic food webs: interactions between two predators and two prey. American Naturalist 135:176-204.

Williams, B.K., T.A.G. Rittenhouse, and R.D. Semlitsch. 2008. Leaf litter input mediates tadpole performance across forest canopy treatments. Oecologia 155:377-384.

Yurewicz, K. L. 2004. A growth/mortality trade-off in larval salamanders and the coexistence of intraguild predators and prey. Oecologia 138:102-111.

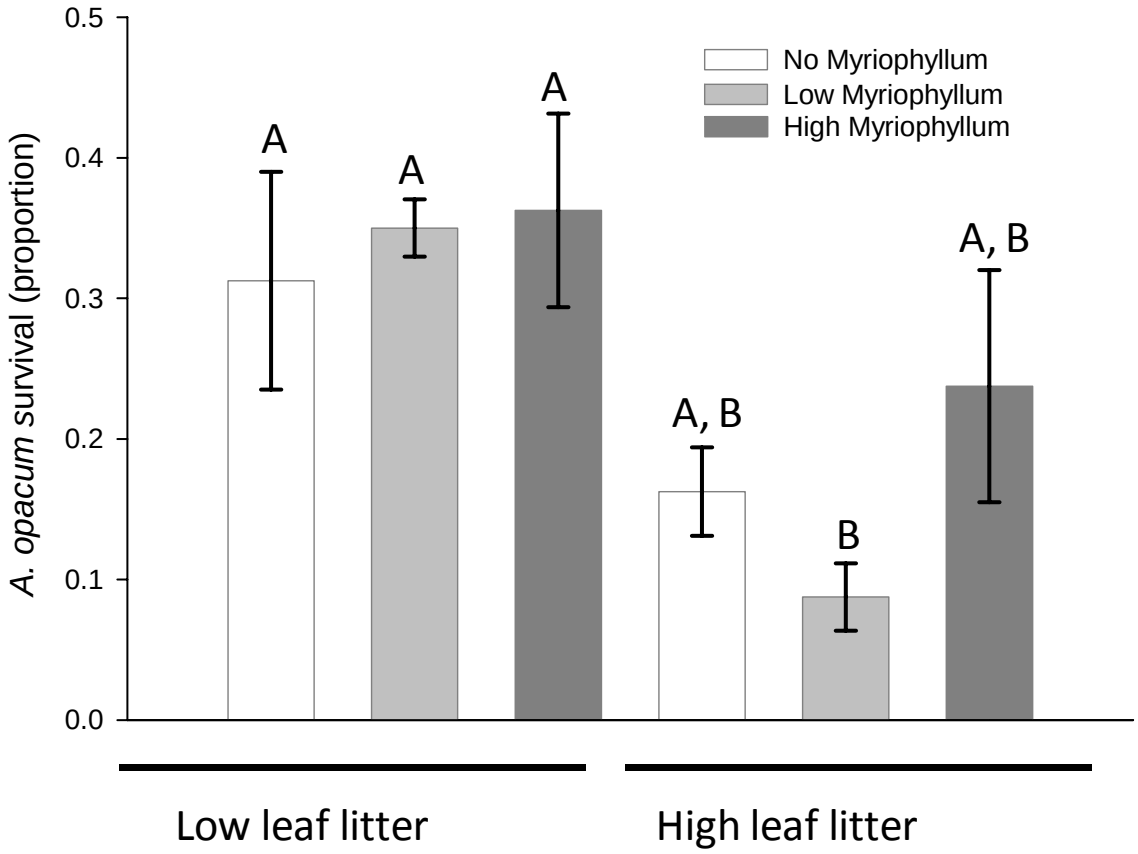


Figure 14. Mean (± 1 SE) survival of *A. opacum* in mesocosms of varying habitat complexity. Means and standard errors are based on values (i.e., proportion of individuals surviving within a pond) associated with an individual pond of a particular treatment. Letters below bars indicate statistical differences among treatments.

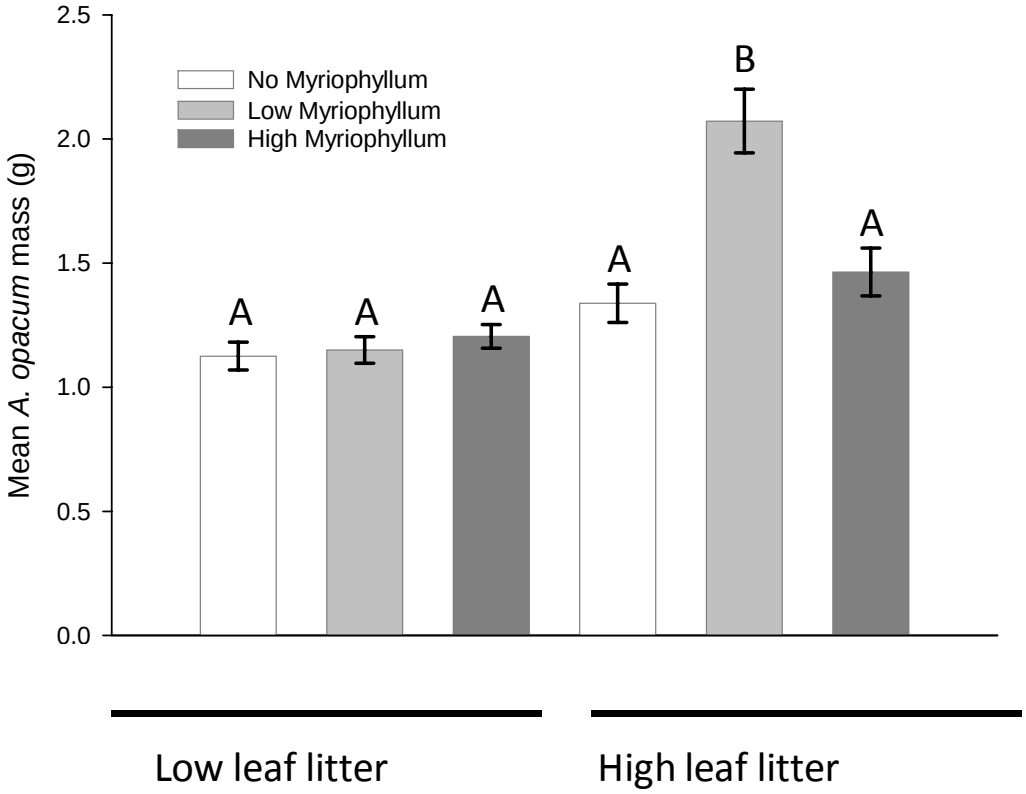


Figure 15. Mean (± 1 SE) mass at metamorphosis of *A. opacum* in mesocosms of varying habitat complexity. Means and standard errors are based on values (i.e., masses of individuals within a pond) associated with an individual pond of a particular treatment. Letters above bars indicate statistical differences among treatments.

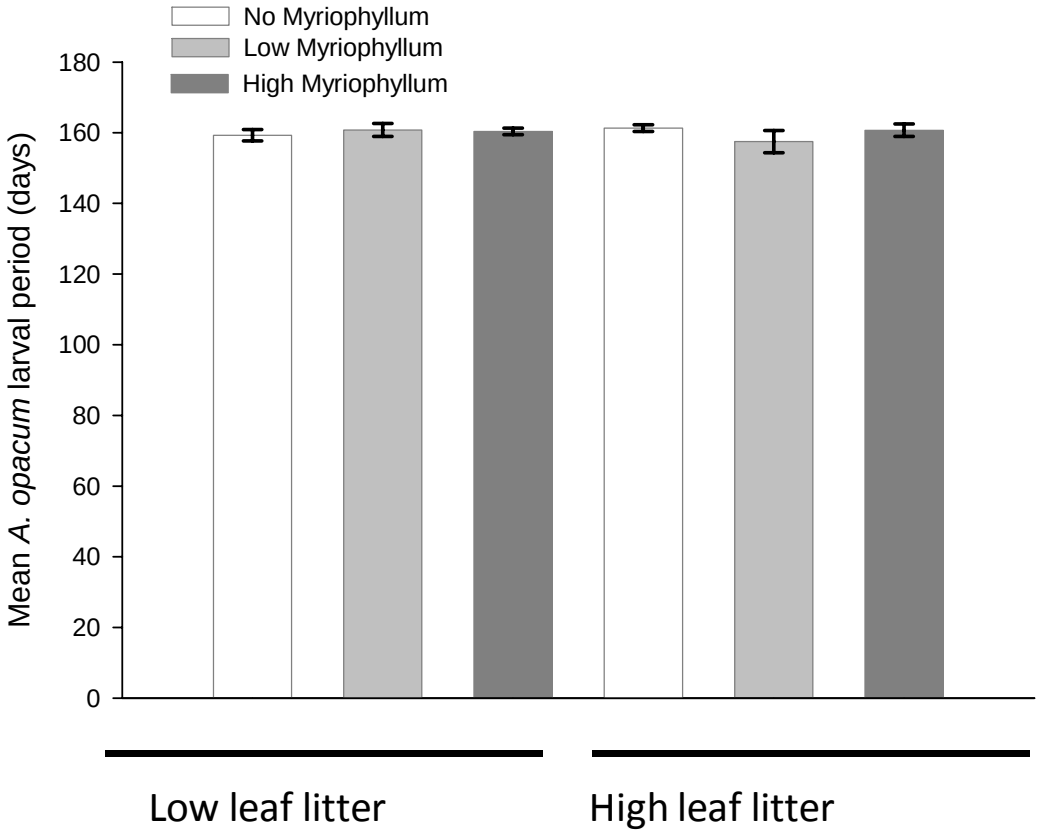


Figure 16. Mean (± 1 SE) larval period of *A. opacum* in mesocosms of varying habitat complexity. Means and standard errors are based on values (i.e., larval period of individuals within a pond) associated with an individual pond of a particular treatment.

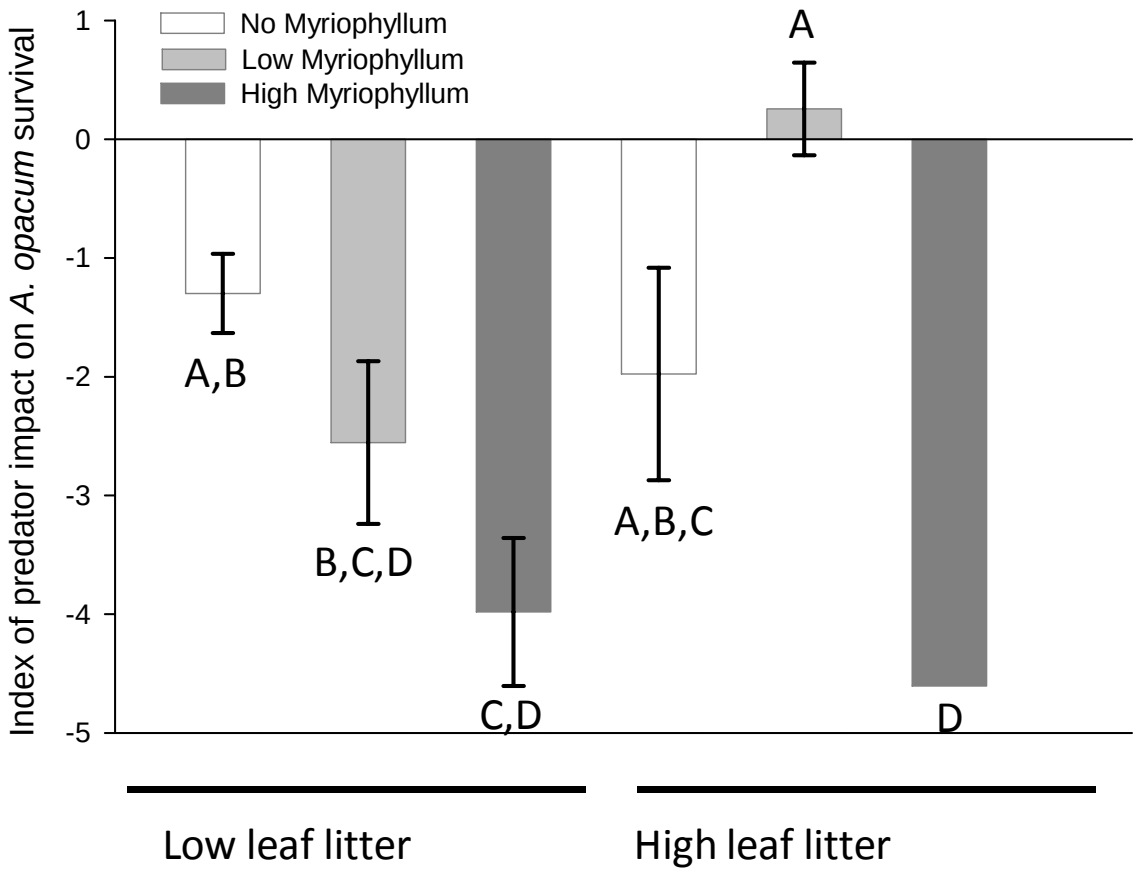


Figure 17. Mean (± 1 S.E.) impact of *Anax* (response with *Anax*/without *Anax*) on *A. opacum* survival in mesocosms varying in habitat complexity. Letters over bars indicate statistical differences among treatments.

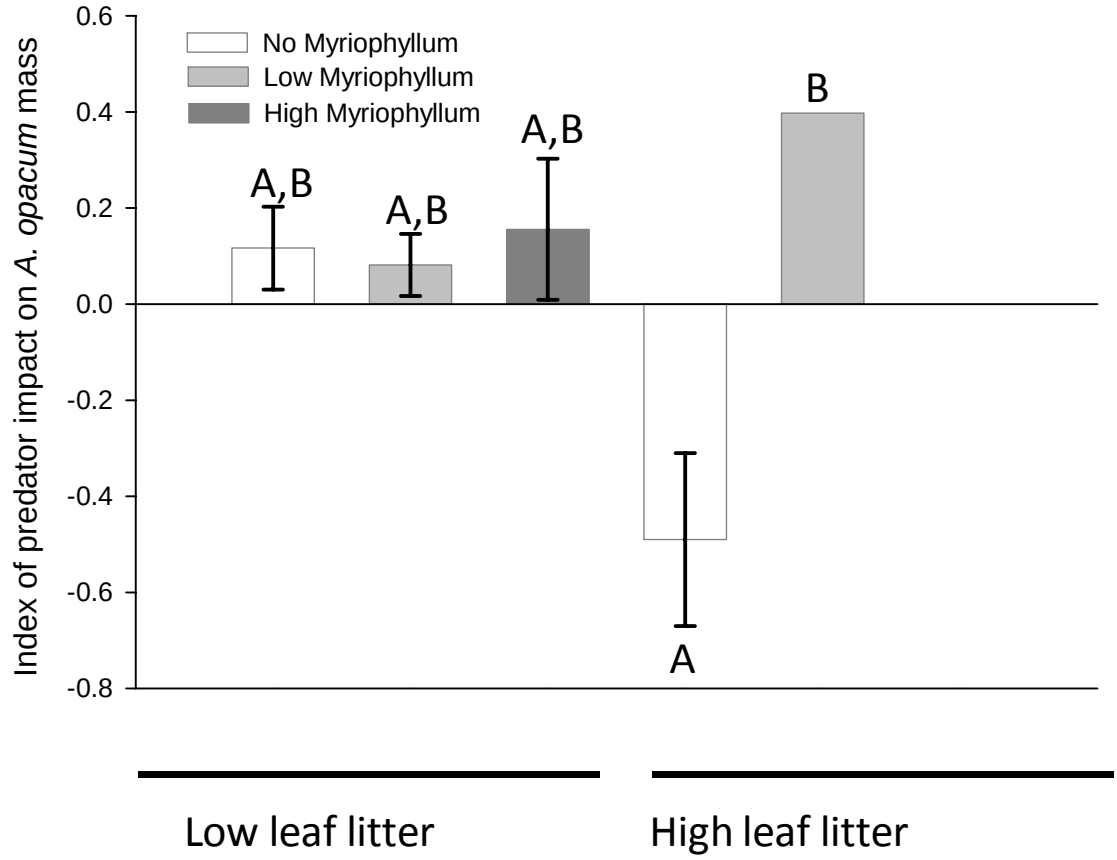
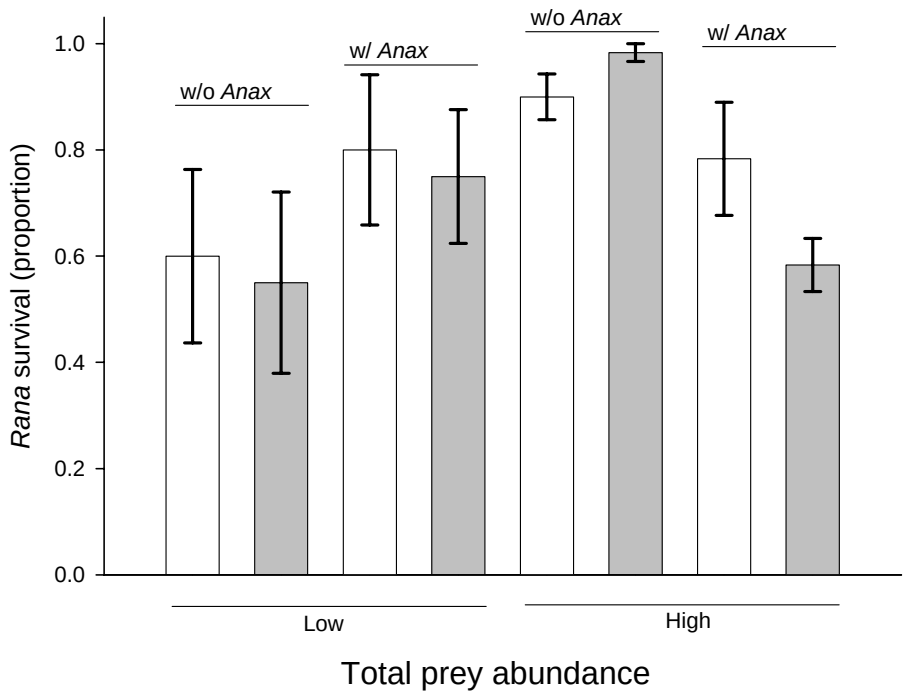


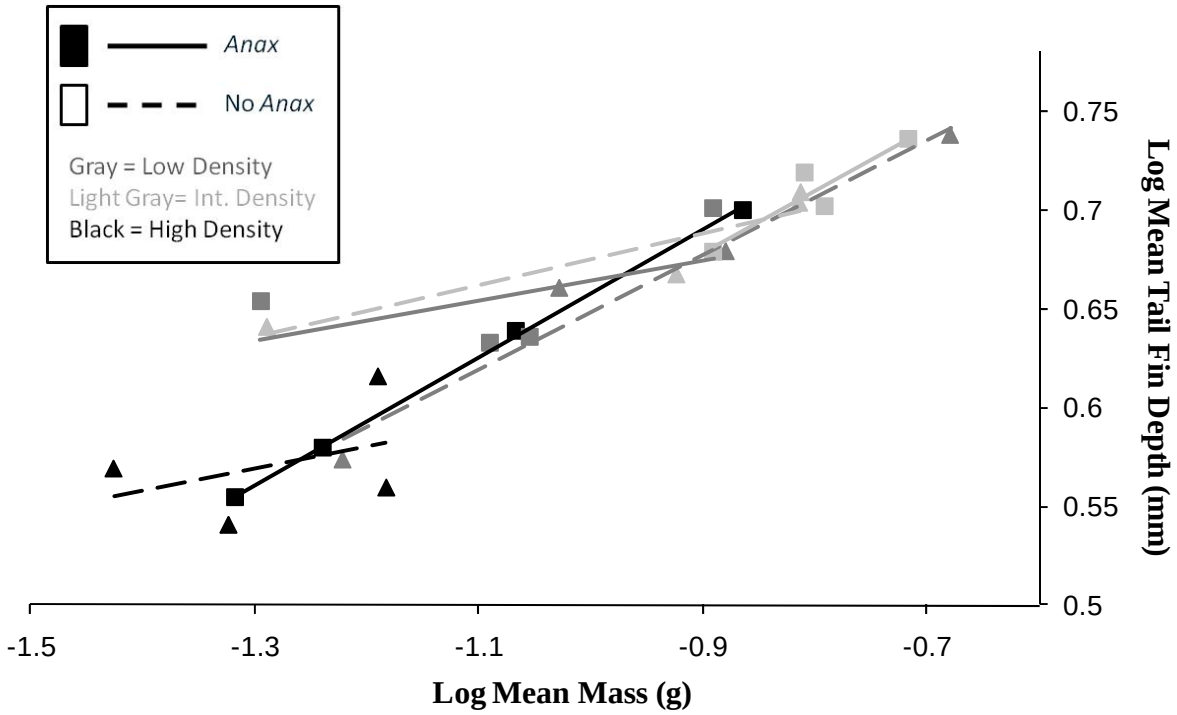
Figure 2. Mean (± 1 S.E.) impact of *Anax* (response with *Anax*/without *Anax*) on *A. opacum* mass at metamorphosis in mesocosms varying in habitat complexity. Letters above bars indicate statistical differences among treatments.

APPENDIX A: CHAPTER 2-Supplemental Figures

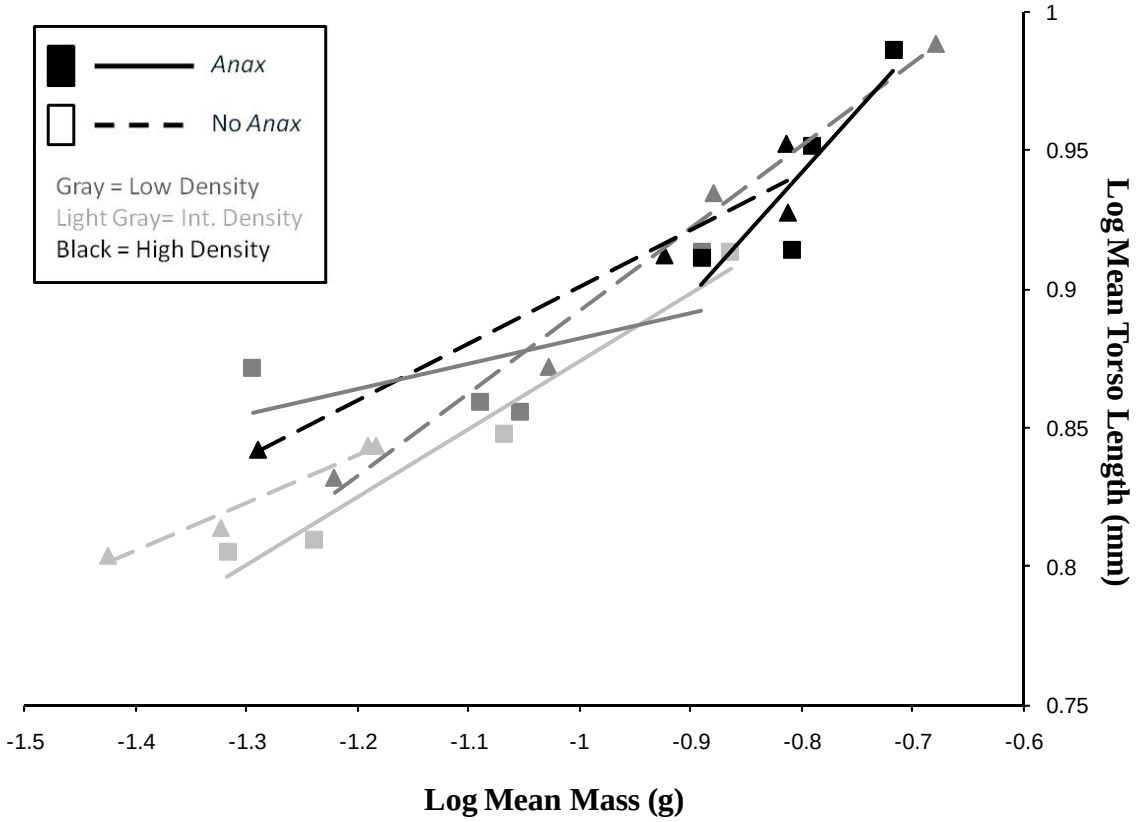


A - 1. Mean proportion of *Rana sphenocephala* survivors (± 1 SE) in eight food webs of differing trophic complexity (presence/removal of the top predator, *Anax*, and presence/removal of shared prey, *Pseudacris crucifer*) and total prey abundance. White bars are food webs without the shared prey, *P. crucifer*, while grey bars are food webs with *P. crucifer* present. Means and standard errors are based on values (i.e., total survival within a pond) associated with an individual pond of a particular treatment. N = 4 in all cases.

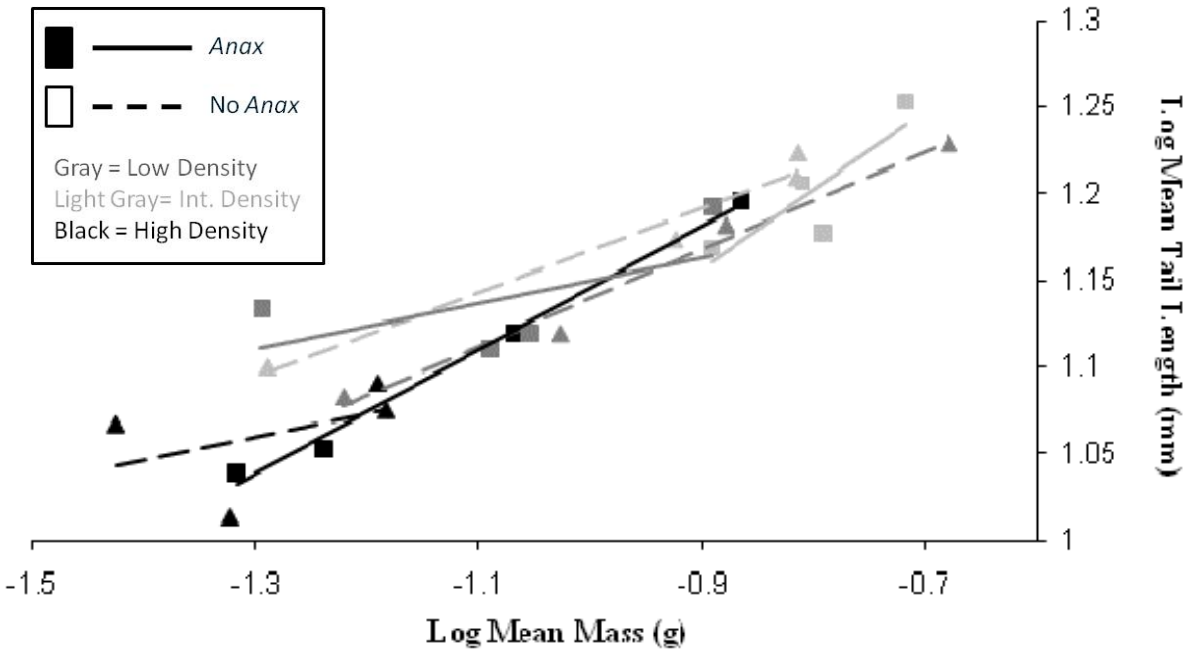
APPENDIX B: CHAPTER 3-Supplemental Figures



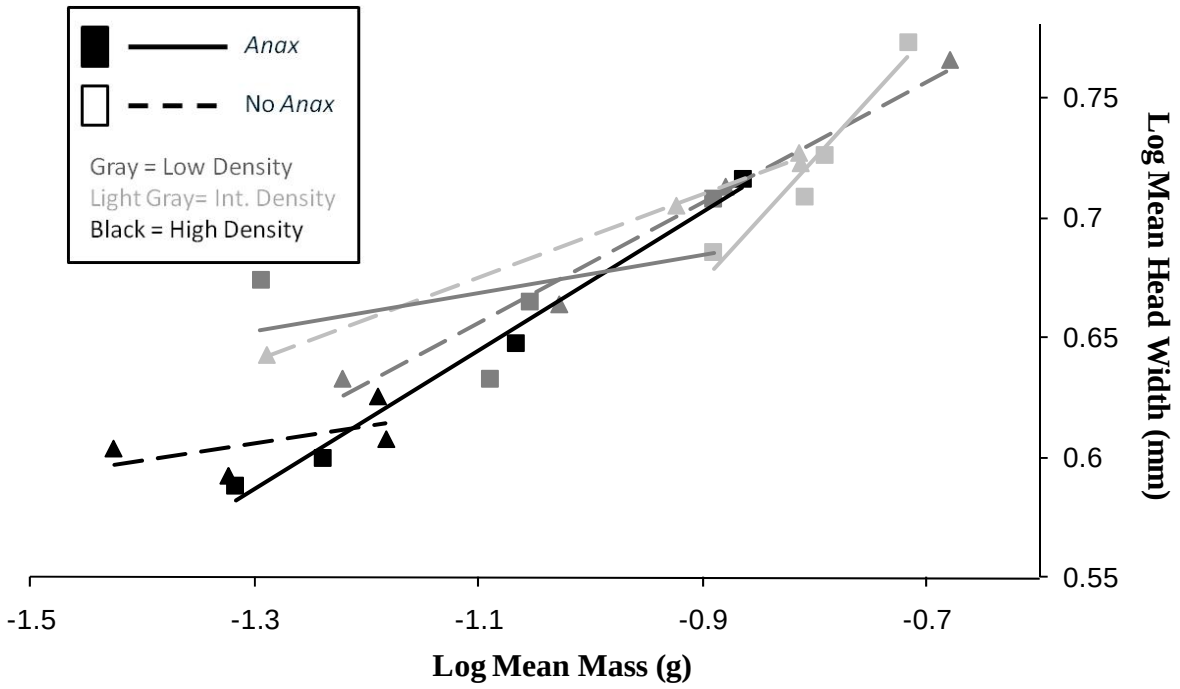
B - 1. Morphological changes in *A. opacum* tail fin depth (corrected for *A. opacum* mass) during the middle sampling period (day 59) for *A. opacum* tail fin depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



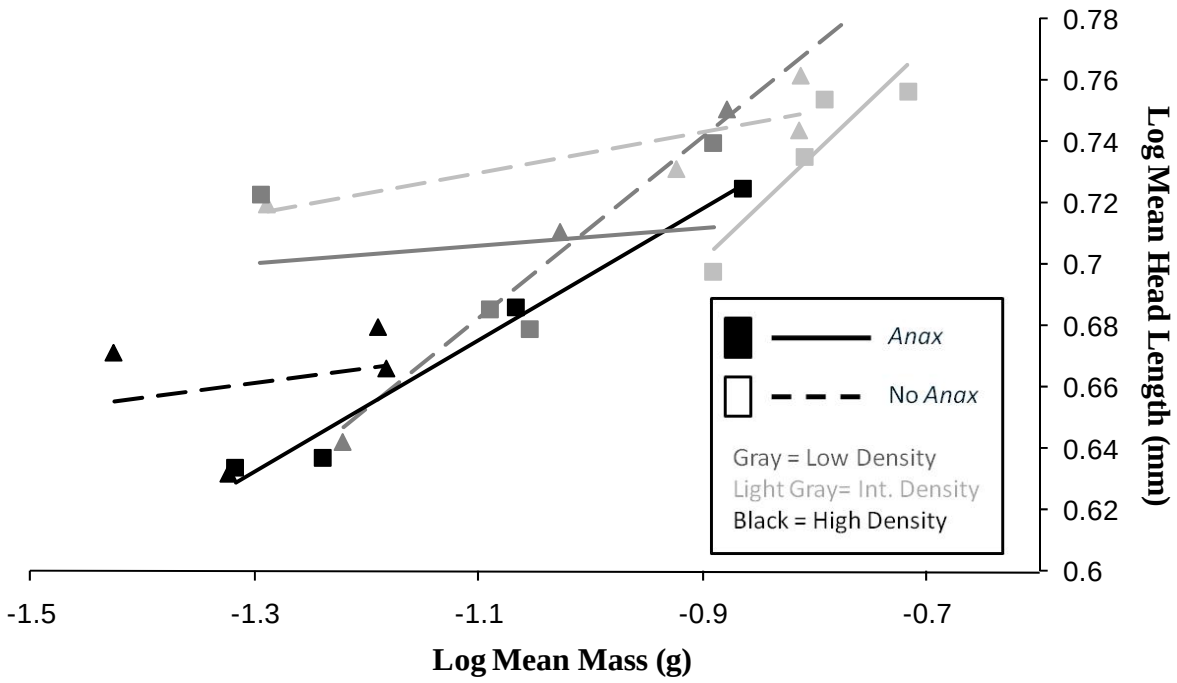
B - 2. Morphological changes in *A. opacum* torso length (corrected for *A. opacum* mass) during the middle sampling period (day 59) for *A. opacum* torso length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



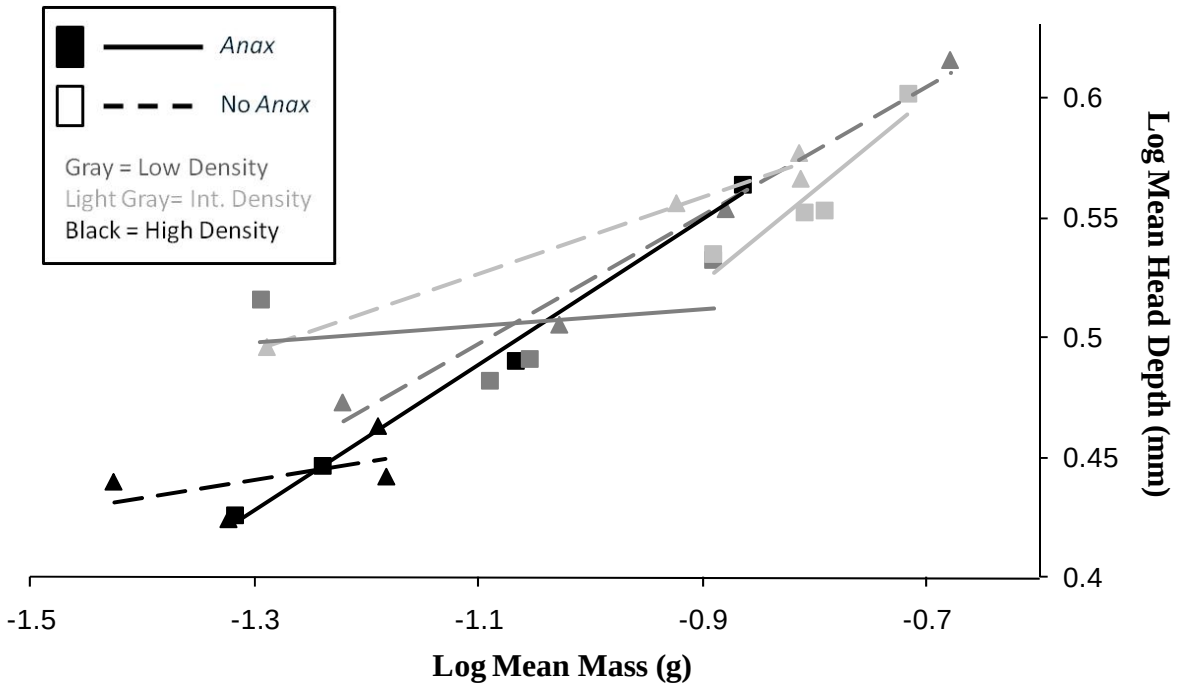
B - 3. Morphological changes in *A. opacum* tail length (corrected for *A. opacum* mass) during the middle sampling period (day 59) for *A. opacum* tail length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



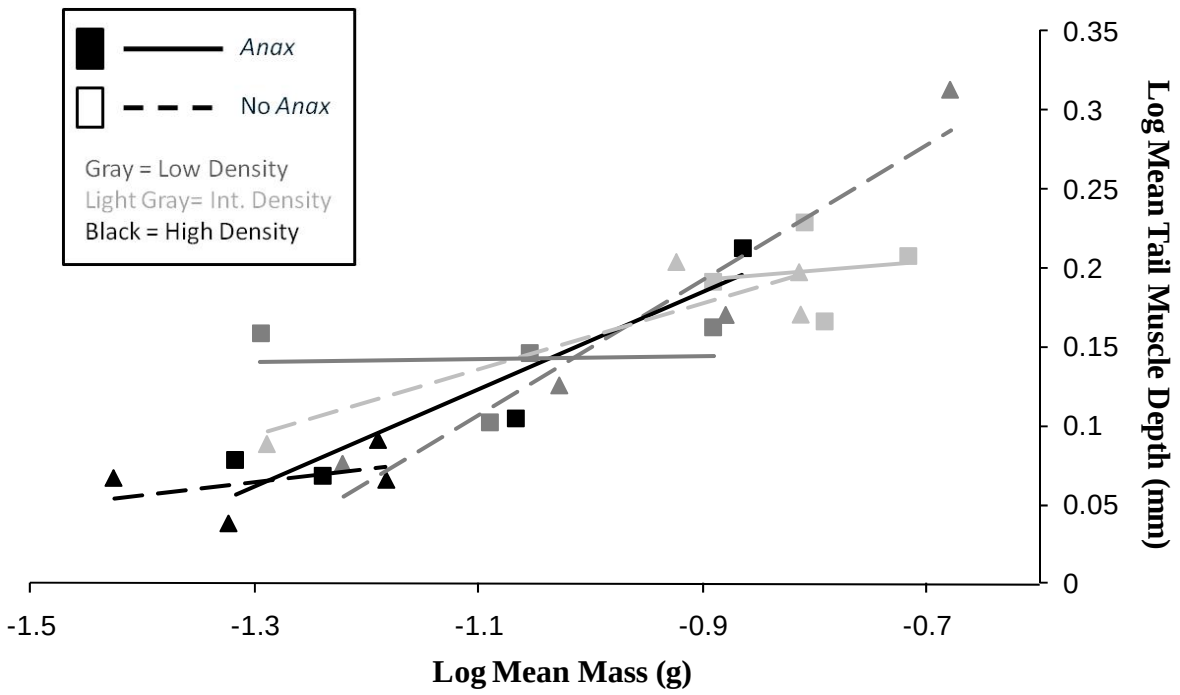
B - 4. Morphological changes in *A. opacum* head width (corrected for *A. opacum* mass) during the middle sampling period (day 59) for *A. opacum* head width. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



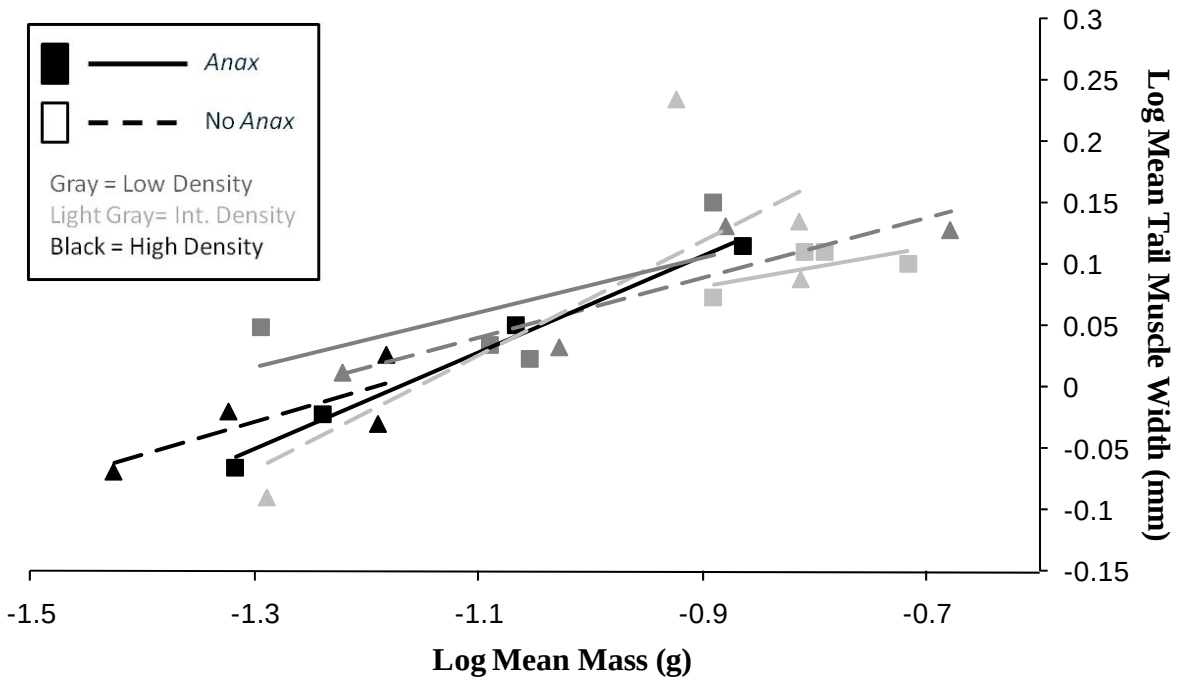
B - 5. Morphological changes in *A. opacum* head length (corrected for *A. opacum* mass) during the middle sampling period (day 59) for *A. opacum* head length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



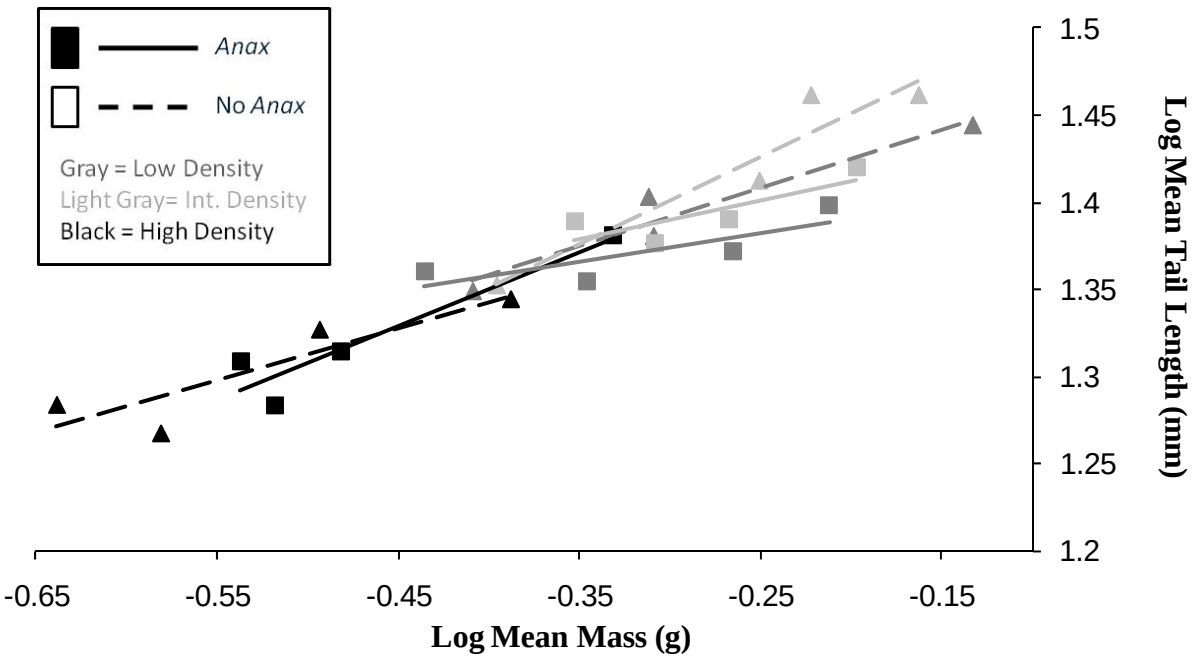
B - 6. Morphological changes in *A. opacum* head depth (corrected for *A. opacum* mass) during the middle sampling period (day 59) for *A. opacum* head depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



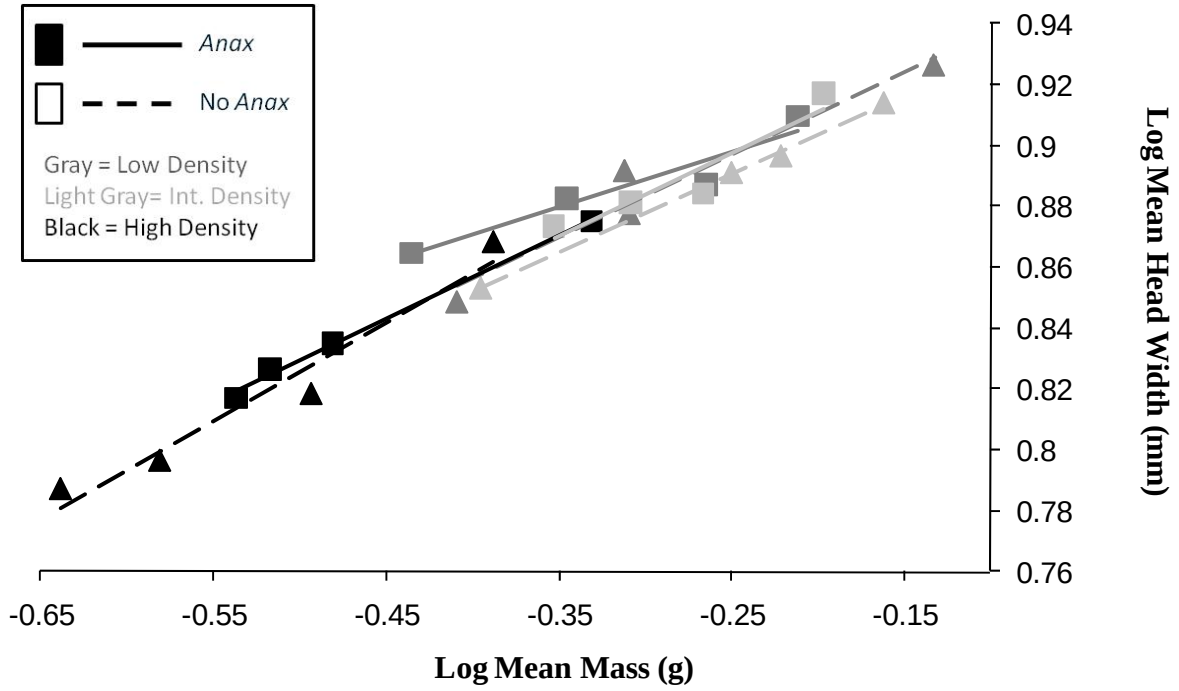
B - 7. Morphological changes in *A. opacum* tail muscle depth (corrected for *A. opacum* mass) during the middle sampling period (day 59) for *A. opacum* tail muscle depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



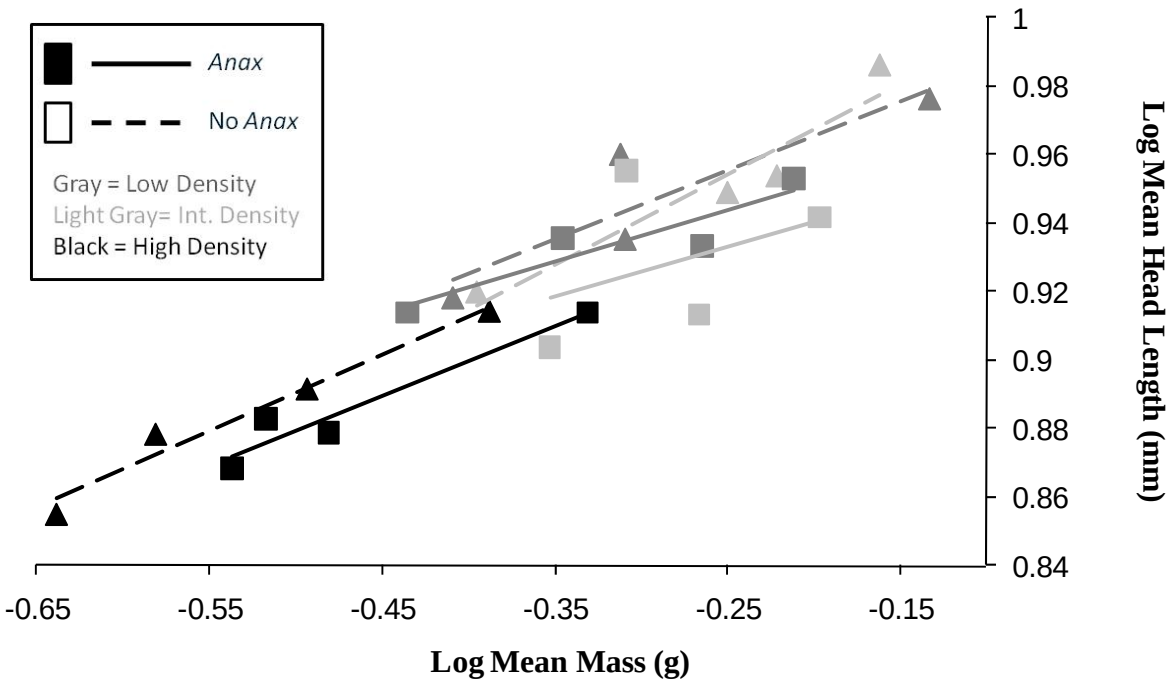
B - 8. Morphological changes in *A. opacum* tail muscle width (corrected for *A. opacum* mass) during the middle sampling period (day 59) for *A. opacum* tail muscle width. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



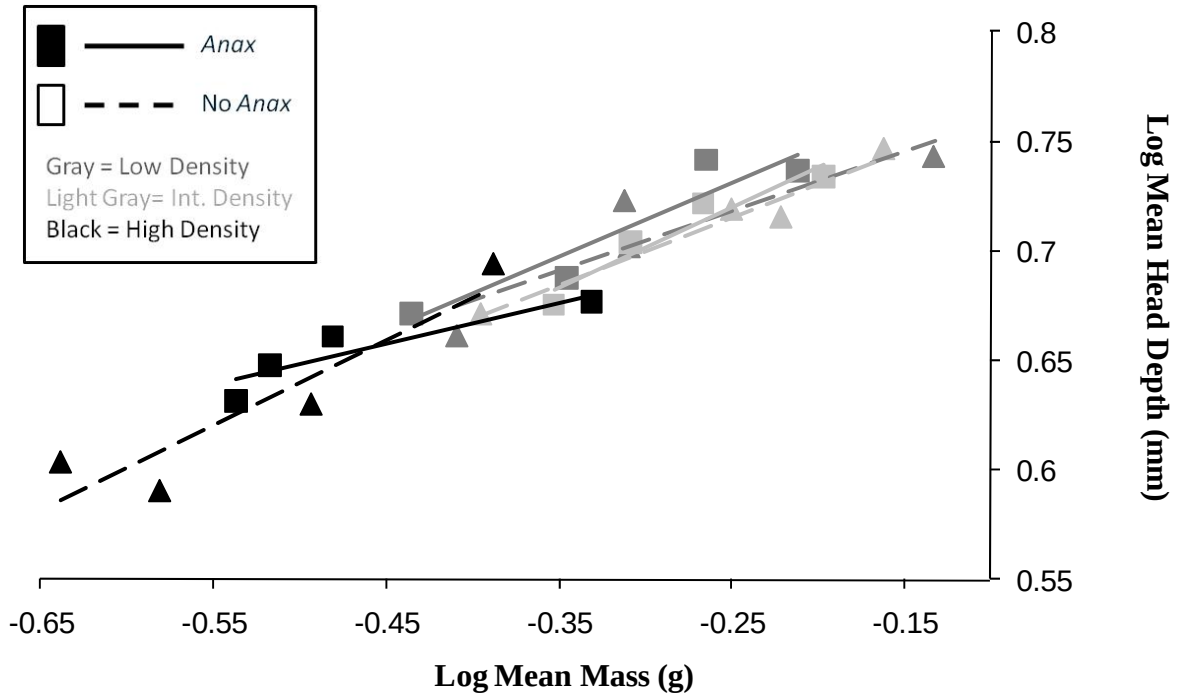
B - 9. Morphological changes in *A. opacum* tail length (corrected for *A. opacum* mass) during the middle sampling period (day 113) for *A. opacum* tail length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



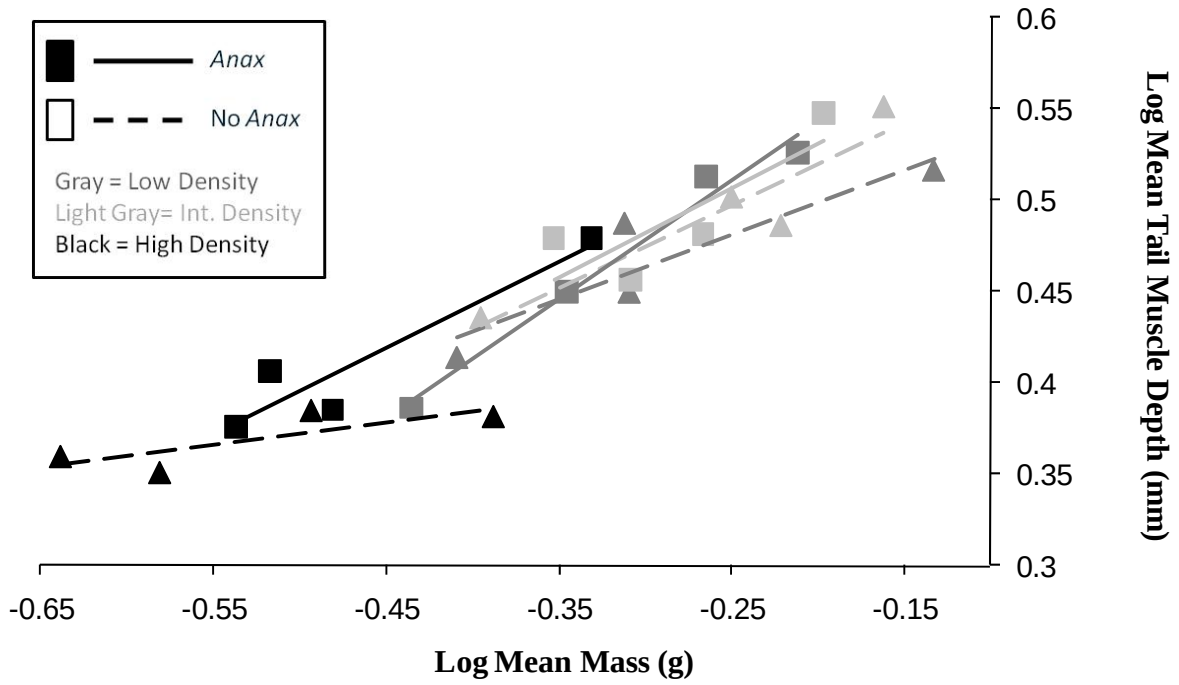
B - 10. Morphological changes in *A. opacum* head width (corrected for *A. opacum* mass) during the middle sampling period (day 113) for *A. opacum* head width. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



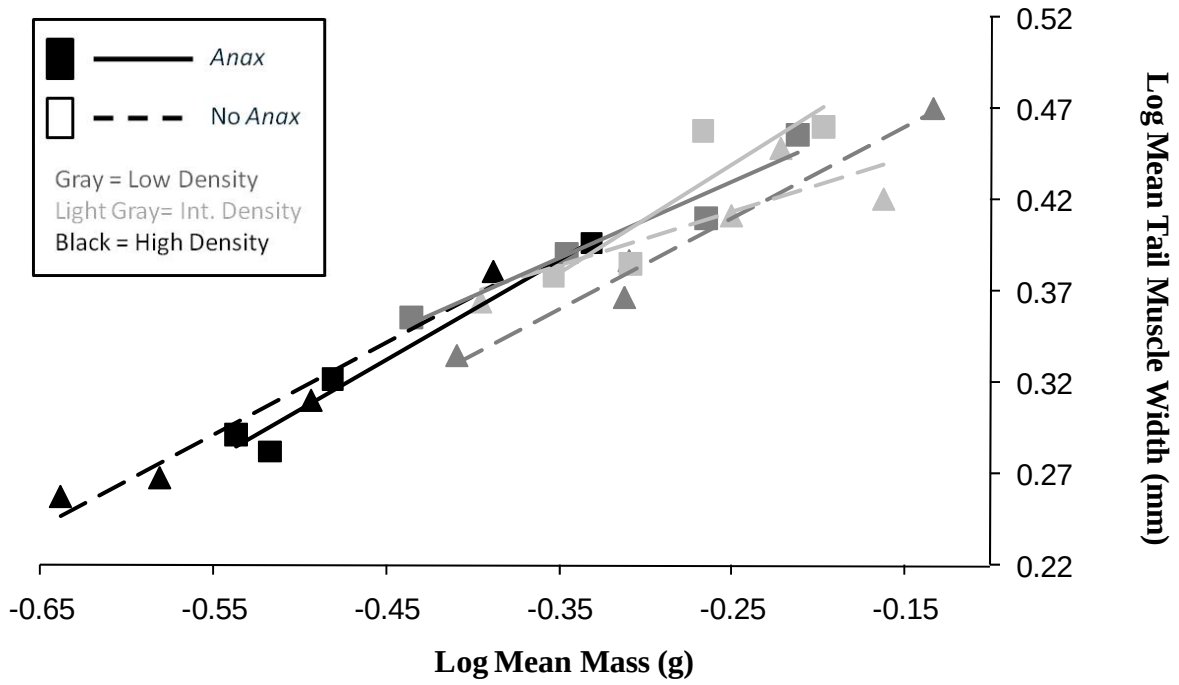
B - 11. Morphological changes in *A. opacum* head length (corrected for *A. opacum* mass) during the middle sampling period (day 113) for *A. opacum* head length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



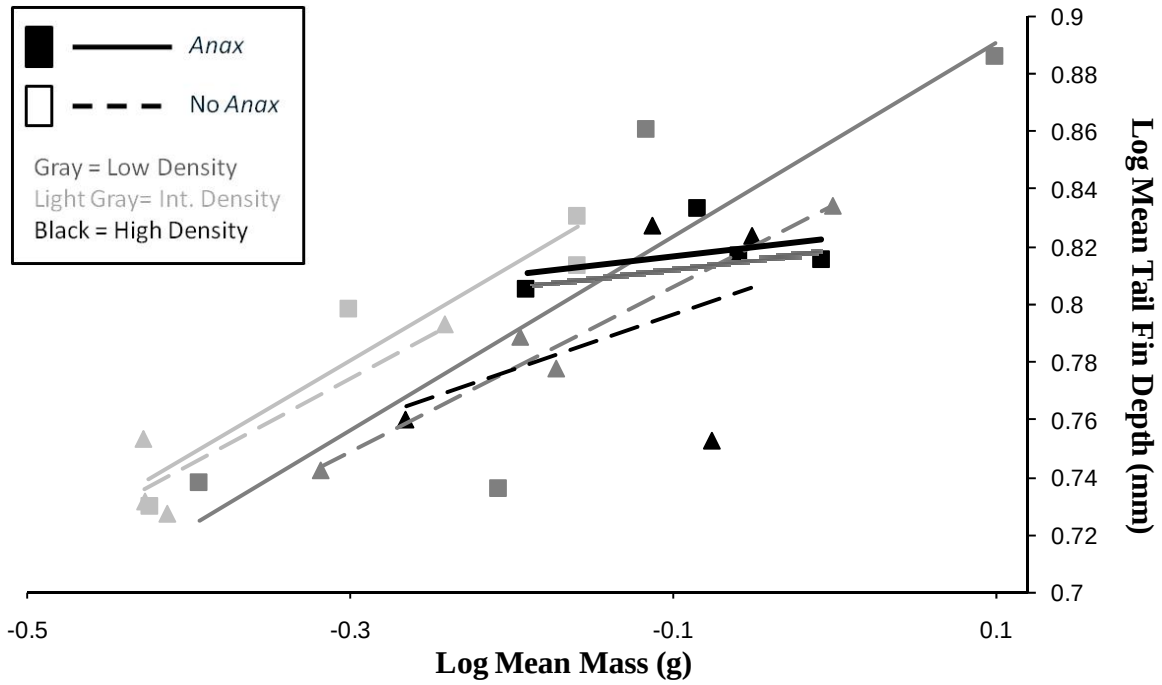
B - 12. Morphological changes in *A. opacum* head depth (corrected for *A. opacum* mass) during the middle sampling period (day 113) for *A. opacum* head depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



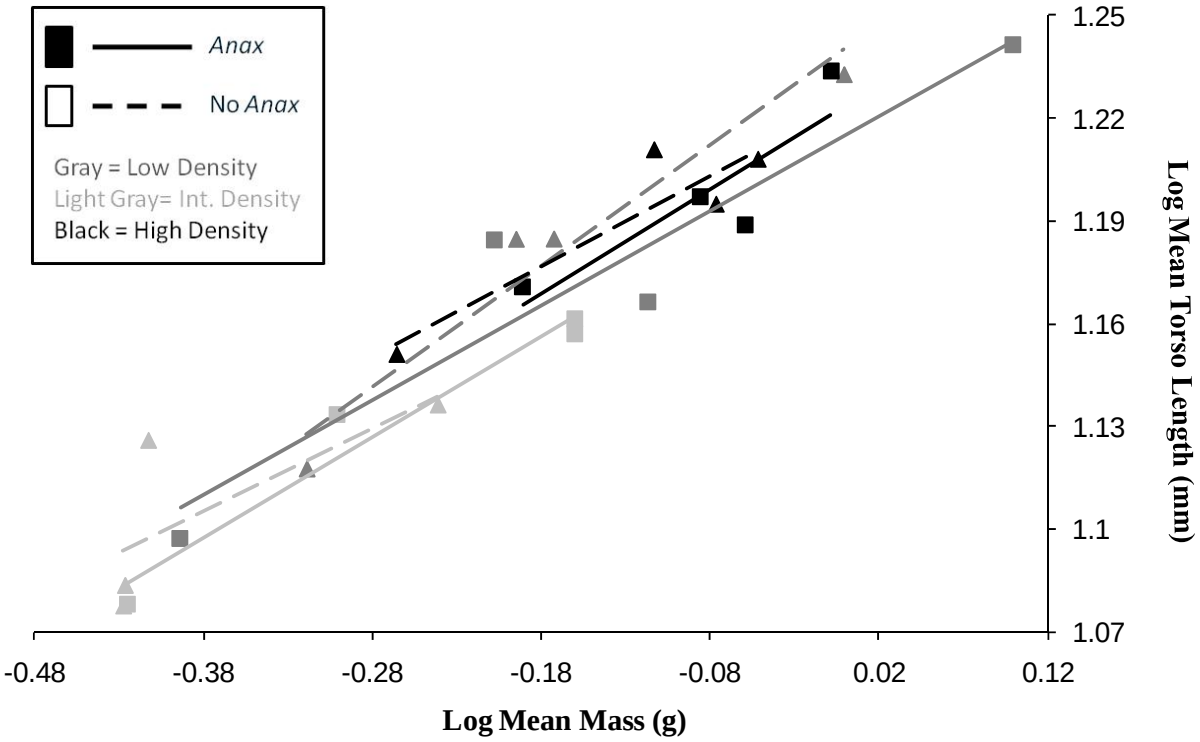
B - 13. Morphological changes in *A. opacum* tail muscle depth (corrected for *A. opacum* mass) during the middle sampling period (day 113) for *A. opacum* tail muscle depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



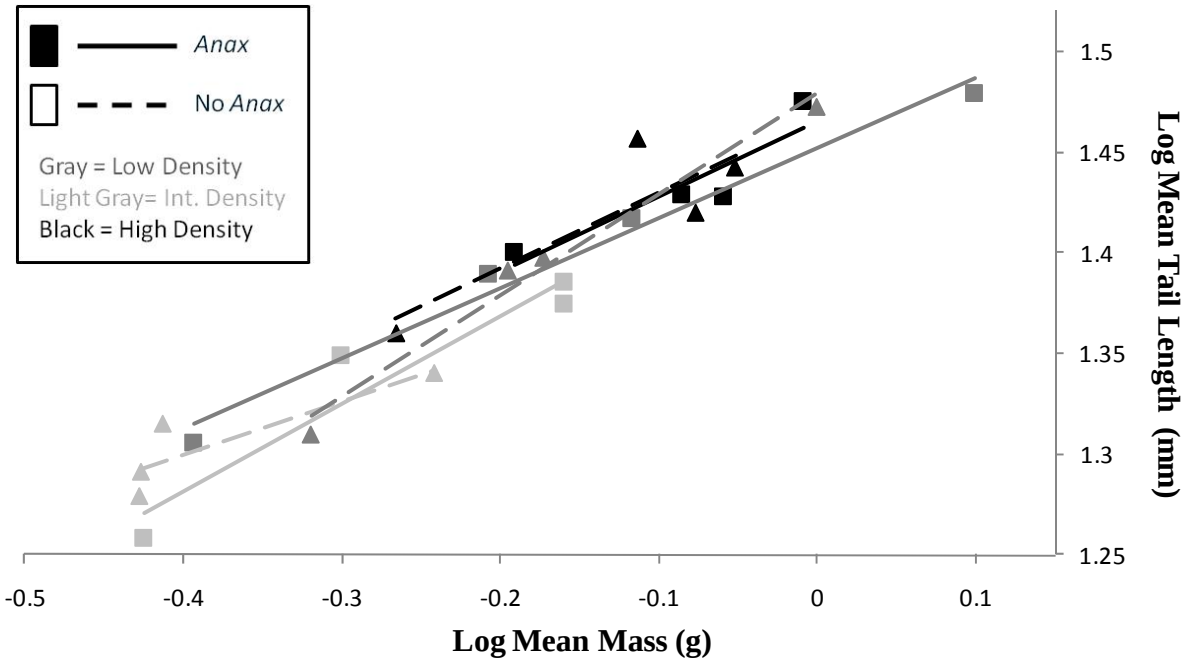
B - 14. Morphological changes in *A. opacum* tail muscle width (corrected for *A. opacum* mass) during the middle sampling period (day 113) for *A. opacum* tail muscle width. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



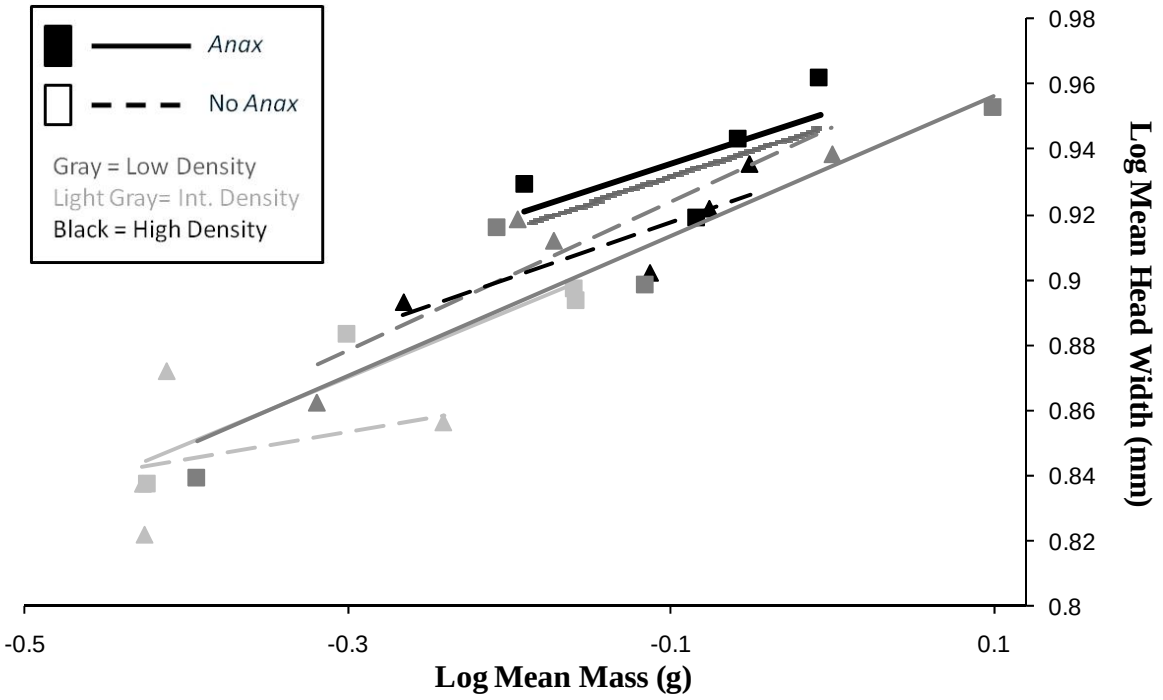
B - 15. Morphological changes in *A. opacum* tail fin depth (corrected for *A. opacum* mass) during the middle sampling period (day 169) for *A. opacum* tail fin depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



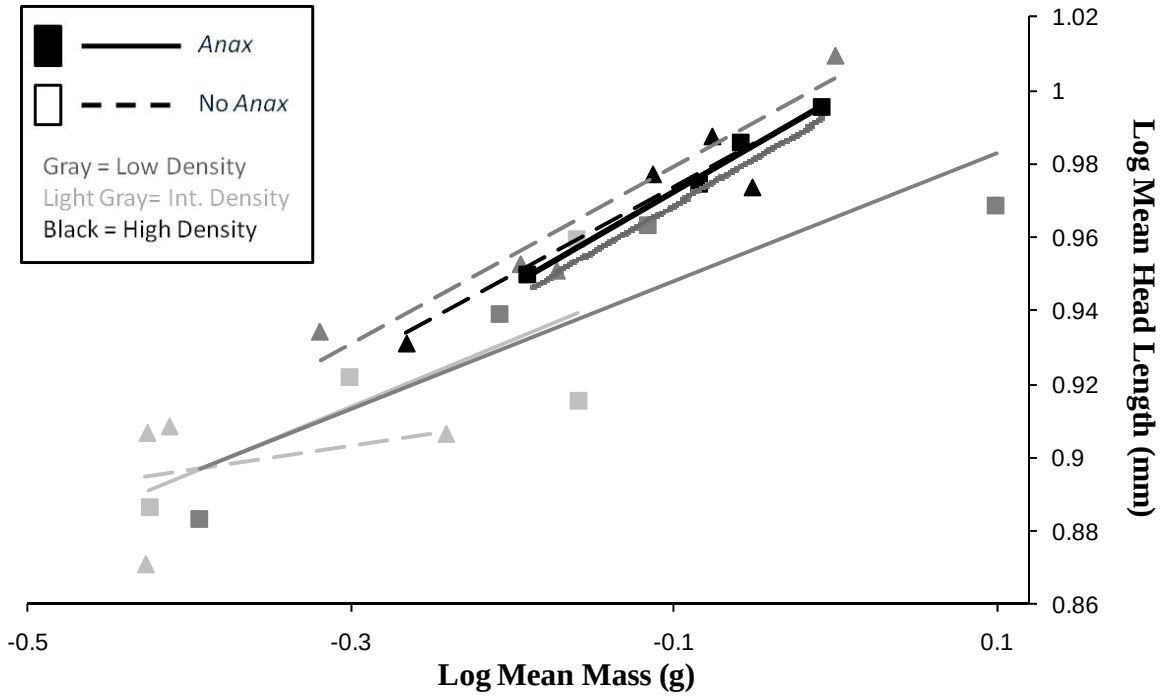
B - 16. Morphological changes in *A. opacum* torso length (corrected for *A. opacum* mass) during the middle sampling period (day 169) for *A. opacum* torso length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



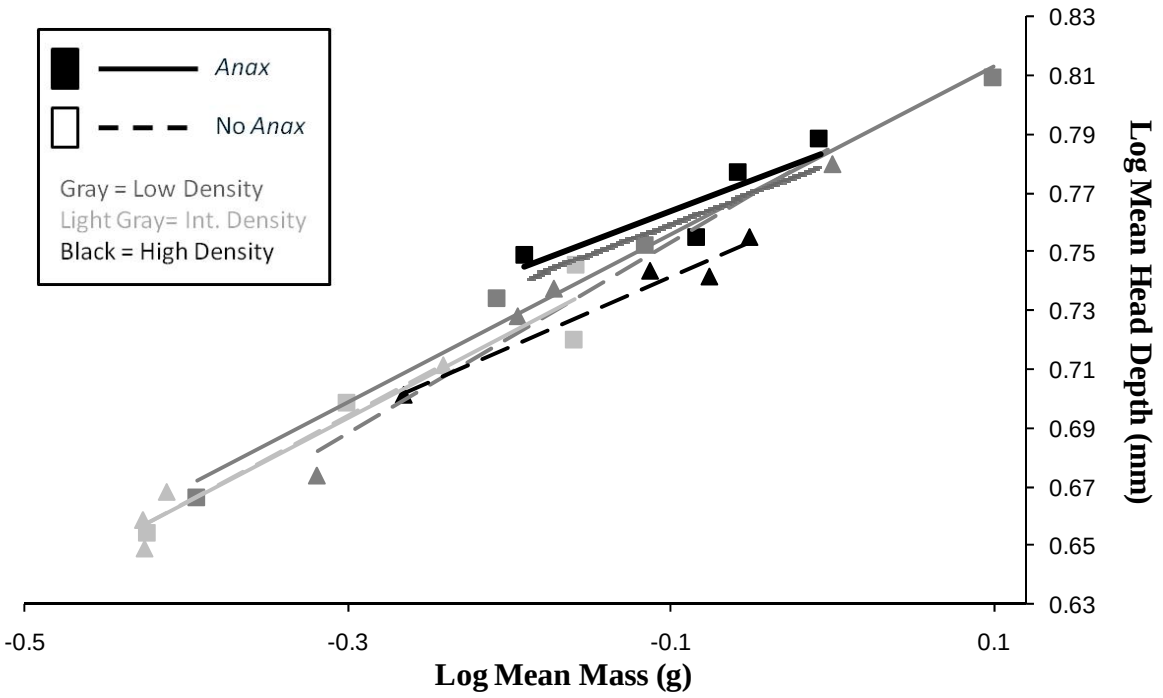
B - 17. Morphological changes in *A. opacum* tail length (corrected for *A. opacum* mass) during the middle sampling period (day 169) for *A. opacum* tail length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



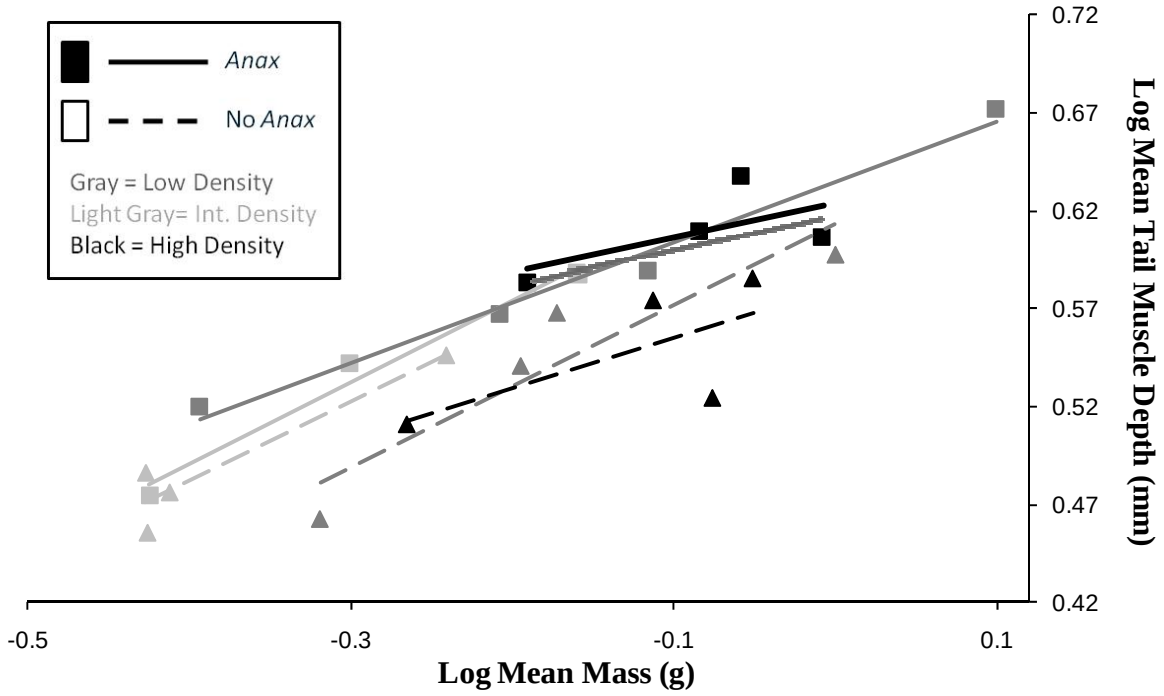
B - 18. Morphological changes in *A. opacum* head width (corrected for *A. opacum* mass) during the middle sampling period (day 169) for *A. opacum* head width. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



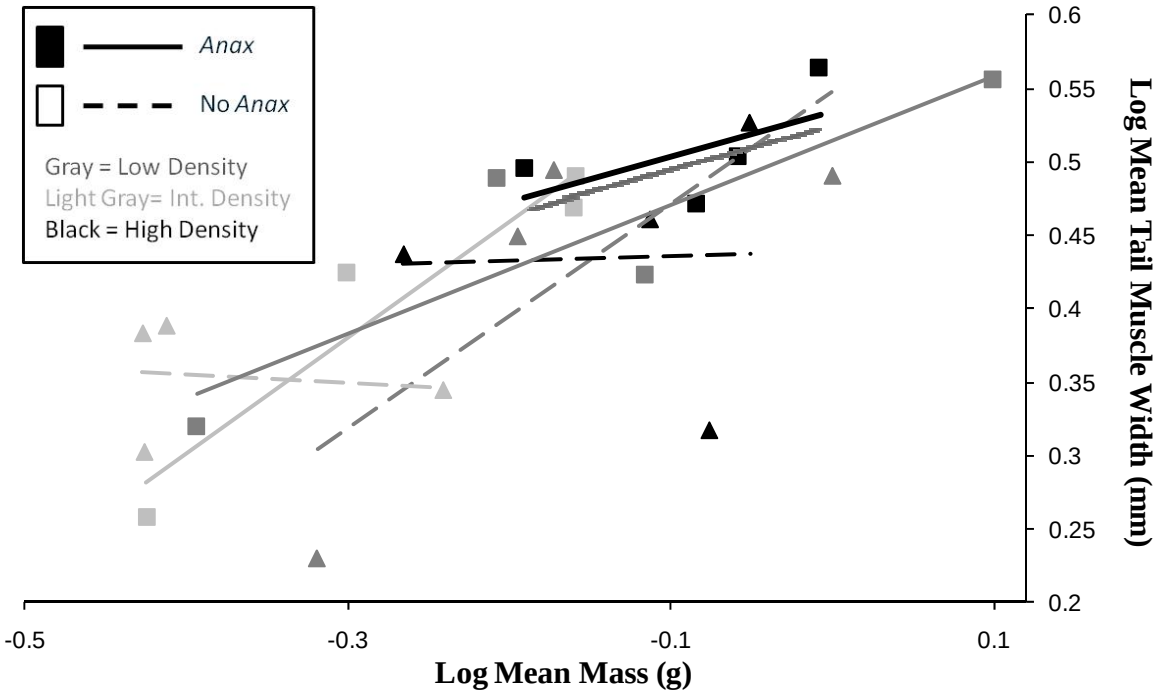
B - 19. Morphological changes in *A. opacum* head length (corrected for *A. opacum* mass) during the middle sampling period (day 169) for *A. opacum* head length. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



B - 20. Morphological changes in *A. opacum* head depth (corrected for *A. opacum* mass) during the middle sampling period (day 169) for *A. opacum* head depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



B - 21. Morphological changes in *A. opacum* tail muscle depth (corrected for *A. opacum* mass) during the middle sampling period (day 169) for *A. opacum* tail muscle depth. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.



B - 22. Morphological changes in *A. opacum* tail muscle width (corrected for *A. opacum* mass) during the middle sampling period (day 169) for *A. opacum* tail muscle width. Squares and solid trendlines are treatments with salamanders exposed to *Anax* cues while triangles and dashed trendlines are treatments with salamanders in the absence of *Anax* cues.

APPENDIX C: Animal Use Protocol Approvals

Animal Care and Use Committee
East Carolina University
212 Ed Warren Life Sciences Building
Greenville, NC 27834
252-744-2436 office • 252-744-2355 fax

March 8, 2007

David Chalcraft, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "Intraguild Predation in Ephermal Pond Communities Involving Larval Salamanders and Dragonflies," (AUP #D209) was reviewed by this institution's Animal Care and Use Committee on 3/8/07. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure

Animal Care and Use Committee
East Carolina University
212 Ed Warren Life Sciences Building
Greenville, NC 27834
252-744-2436 office • 252-744-2355 fax

November 5, 2007

David Chalcraft, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "Effects of Dragonfly Naiads and Intraspecific Abundance on the Morphology and Behavior of Larval Salamanders," (AUP #D218) was reviewed by this institution's Animal Care and Use Committee on October 30, 2007. The following action was taken by the Committee:

"Approved"

In addition, the following are informational comments (no response needed):

1. Is the food source sufficient enough to sustain the dragonfly larvae that are placed into tanks with a limited number of salamander larvae?
2. Do you have an SOP for the maintenance of the artificial ponds? If not, you may want to develop one so that you can simply refer to it in new AUPs rather than describing the set-up each time.
3. Your laboratory will need to be approved as a holding facility.

Please contact me if I can be of further assistance.

Sincerely yours,

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure

Animal Care and Use Committee
East Carolina University
212 Ed Warren Life Sciences Building
Greenville, NC 27834
252-744-2436 office • 252-744-2355 fax

December 11, 2008

David Chalcraft, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "Effects of Habitat Complexity on Interactions Among Dragonfly Naiads and Larval Salamanders," (AUP #D227) was reviewed by this institution's Animal Care and Use Committee on 12/11/08. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure

East Carolina University

1907-2007
CENTENNIAL

Animal Care and Use Committee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

February 11, 2010

David Chalcraft, Ph.D.
Department Biology
Howell Science Complex
East Carolina University

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "Costs and/or Benefits of Predator- and Competitor-Induced Phenotypic Plasticity in Larval Marbled Salamanders," (AUP #D242) was reviewed by this institution's Animal Care and Use Committee on 2/11/10. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Robert G Carroll, Ph.D

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure